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REVIEW

New Treatment Options for Lung Adenocarcinoma - in View of Molecular Background

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Abstract Lung cancer is the leading cause of cancer related mortality all over the world, and a number of developments have indicated future clinical benefit recently. The development of molecular pathology methods has become increasingly important in the prediction of chemotherapy sensitivity and mutation analysis to identify driver mutations as important targets of new therapeutic agents. The most significant changes in the treatment of NSCLC revealed in new pathologic classification and in the introduction of molecularly targeted therapies, which include monoclonal antibodies and small molecule tyrosine kinase inhibitors. The side effects of these agents are generally better tolerated than those of conventional chemotherapy and show higher efficacy. The most important factor follows: histology subtypes, gene mutation status, patients' selection, drug toxicities and occurence of drug resistance. In the advanced disease, the hope of cure is less than 3 %, but improvements in survival have been clearly achieved. Some years ago the median lung cancer survival rate was 10–12 months, now in case of available specific molecular targets, a significant increase in median survival rates to 24–36 months has been achieved. These agents give an opportunity to provide a new standard of care. Therefore testing EGFR mutations and ALK rearrangements in patients with advanced lung adenocarcinoma should be incorporated into routine clinical practice. This review focuses on the rationale for targeted agents and new treatment possibilities in case of advanced lung adenocarcinoma.

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Keywords Non-small cell lung cancer (NSCLC) · Adenocarcinoma · Targeted therapies · Signal transduction pathway · Tyrosine kinase inhibitors · Monoclonal antibodies · EGFR mutation · KRAS · EML-4 ALK · VEGFR

Abbreviations

NSCLC	Non-small cell lung cancer
SCLC	Small cell lung cancer
NOS	Not otherwise specified
EGFR	Epidermal growth factor receptor
VEGFR	Vascular endothelial growth factor receptor
PDGFR	Platelet-derived growth factor receptor
ALK	Anaplastic lymphoma kinase
TKI	Tyrosine kinase inhibitor
PFS	Progression free survival
OS	Overall survival
ORR	Overall response rate
QOL	Quality of life

New Pathologic Classification of Lung Adenocarcinoma

A significant changes in pathologic classification of lung cancer was published by the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (AST) and the European Respiratory Society (ERS) in 2011 [1]. It was recognized that 70 % of patients with lung cancer present with advanced disease, which is usually diagnosed on the basis of small biopsies and cytology. Only 30 % of patients, who had undergone surgical resections, had resection specimens. Therefore the new pathological classification is divided into two components: 1: small biopsy and cytology specimens for patients with advanced-stage lung cancer, and 2: resection specimens for operable patients who are eligible

for surgical resection. The main reason for this classification is based on to achieve tumor specific treatment in advanced lung cancer by the determination of correct histology and genetics alterations.

If an NSCLC does not show definitive glandular or squamous morphology in a small biopsy or cytology specimen, it is classified as NSCLC-NOS (Table 1).

The 5-year disease –free survival rate among patients who have minimal invasive adenocarcinoma should be near 100 % if the lesion is completely resected. Invasive adenocarcinoma represents more than 70 % to 90 % of surgical resected lung carcinomas. These tumors consist of a complex heterogeneous molecular pathological caracterisctics of biological patterns. The subtypes are now classified according to the predominant component. EGFR mutations and ALK rearrangements are almost exclusively seen in lung adenocarcinoma, and the identification of these molecular abnormalities is clinically relevant [2]. The frequent finding of KRAS mutation and lack of EGFR mutation in invasive mucinous adenocarcinoma is the most frequent histologic correlation.

Table 1 New histopathology classification of lung adenocarcinoma [1]

IASLC/ATS/ERS Terminology for lungadenocarcinoma, in small biopsies and cytology	IASLC/ATS/ERS Terminology of lung adenocarcinoma in resection specimens
–Mixed subtype	Preinvasive Lesions
–Acinar	Atypical adenomatous hyperplasia
–Papillary	Adenocarcinoma in situ
–Solid	($\leq 3\,cm$, formerly solitary BAC)
–Lepidic (non mucinous)	Non mucinous
–Lepidic (mucinous)	Mucinous
	Mixed mucinous/nonmucinous
	Minimally invasive adenocarcinoma ($\leq 3\,cm$ lepidic predominant tumor with $\leq 5\,mm$ invasion)
	Non mucinous
	Mucinous
	Mixed mucinous/non mucinous
	Invasive adenocarcinoma
	Lepidic predominant
	Acinar predominant
	Papillary predominant
	Micropapillary predominant
	Solid predominant with mucin production
	Variants of invasive adenocarcinoma
	Invasive mucinous adenocarcinoma (including formerly mucinous BAC)
	Colloid
	Fetal (low and high grade)
	Enteric

Non-small-cell lung cancers have been classified according to histopathological features. Adenocarcinoma is classified various number of driver mutations. The mutations are mutually exclusive, except for those in *PIK3CA*.

Therapeutic Possibilities for Lung Adenocarcinoma

Lung cancer came in the forefront of clinical research in the 1990's due to poor prognosis and the large number of patients. The present standard cytotoxic treatment for advanced NSCLC is a combination chemotherapy – a combination of cisplatin or carboplatin and other active chemotherapeutic agents (paclitaxel, docetaxel, gemcitabin, vinorelbin, pemetrexed) [3]. A meta-analysis of 16 randomized controlled trials, involving 2714 patients with systemic chemotherapy versus best supportive care alone, a statistically significant improvement in overall survival (OS) with chemotherapy was reported [4]. Current standard of care for first line therapy in stage III/B- IV involves the use of combination chemotherapy regimen, usually including cisplatin or carboplatin plus another active agent [5]. The overall response rates (RR) of the third –generation regimen used in these trials ranged from 19 % to 32 %, with median survivals of 8–14 months [6]. Standard platinum based doublet chemotherapeutic treatment of advanced NSCLC seems to have reached a plateau in terms of efficacy. The PARAMOUNT trial has confirmed the survival advantage of continuous maintenance therapy with pemetrexed in non-squamous subtype [7]. This maintenance therapy with pemetrexed significantly reduced the risk of disease progression in patients with advanced NSCLC who had not progressed during pemetrexed/cisplatin induction [8].

Molecular Target Guided Treatment

In the past NSCLC were seen together without paying attention to the more specific molecular pathological types. This was accepted, because there were not different therapeutic procedures that could have been applicable for the treatment of these subtypes such as adenocarcinoma and squamous cell carcinoma. It has changed in 2004 when a special EGFR mutation was identified, and nowadays this rapidly evolving field gives new and new results.

The driver mutations occur in genes that encode signalling proteins crucial for cellular proliferation and survival. Mutant oncogenes not only drive tumor formation but also mainten. Understanding the molecular background of solid tumours and an increase in the efficiency of therapy have encouraged researchers to investigate the molecular background of lung cancer. As part of this research strategy, various specific "driver mutations" have been identified for NSCLC (Fig. 1 and Table 2).

Combining targeted agents may address to destroye the resistance observed with some single target agents and may

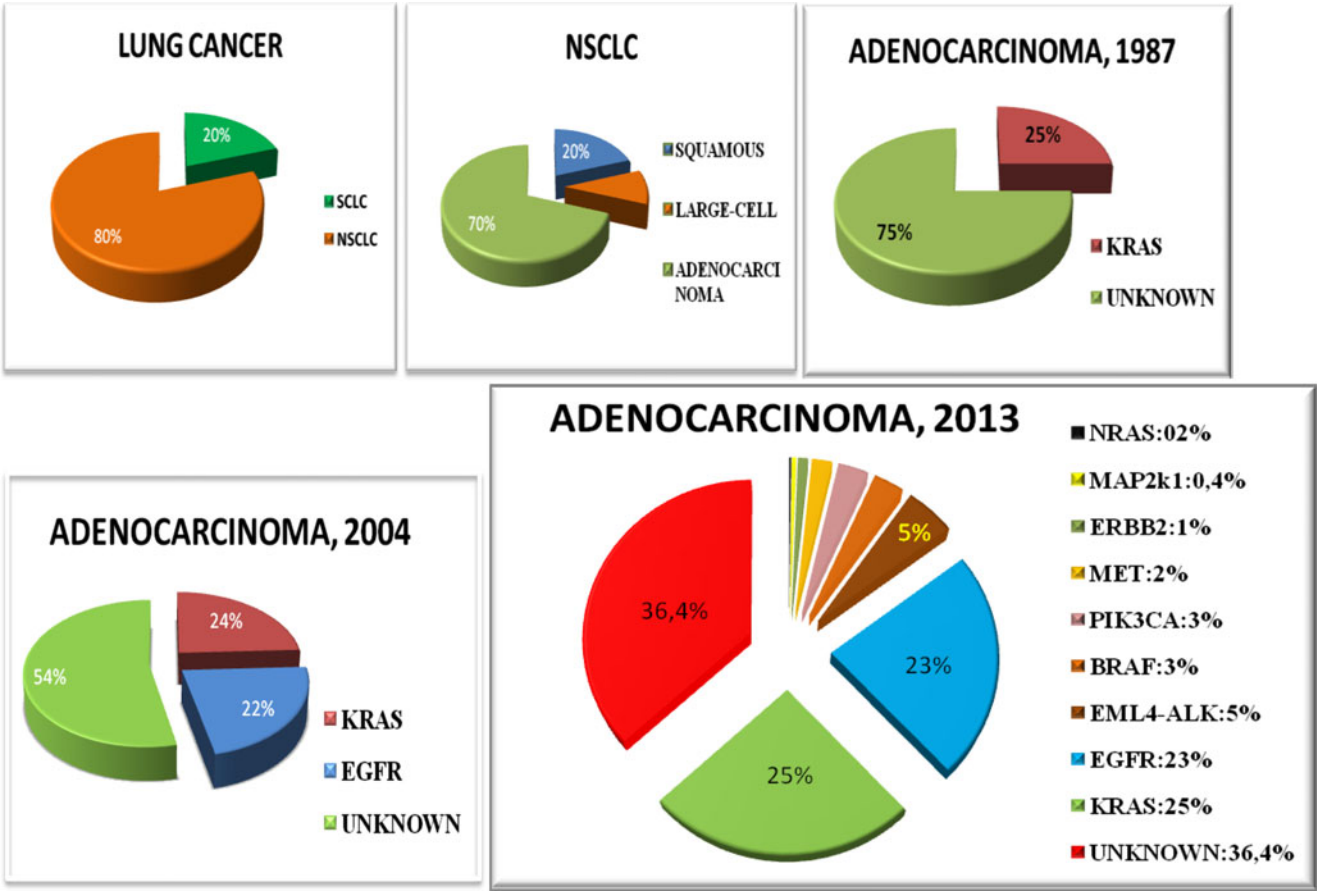

Fig. 1 Evolution of mutation status in non-small-cell lung cancer

produce additive or synergistic effects influencing angiogenesis,apoptosis, tumorigenesis and tumor growth [9]. The combined treatment options are determined by the clinical condition of the patient and the molecular pathological profiles. The divergent toxicity is also worthy of special attention.

Table 2 Frequency of occurrence of individual mutations in the case of NSCLC [10]

Types of mutations	Adenocarcinoma	Squamous cell carcinoma
EGFR	Asians: 30–50 % Whites: 10 %	0–3 %
ALK	5–7 %	1 %
HER2	2.8 %	0
BRAF	2–5 %	0
KRAS	Asians: 10 % Whites: 30 %	1 %
PIK3CA	<5 %	3.6–6.5 %
AKT1	0	<5 %
MAP2K1	<5 %	0
MET	<5 %	<5 %
RET	1.2–1.9 %	0
ROS1	1.2–2.6 %	0
FGFR1	3.4 %	9.7–21 %
ERBB2	1 %	0
NRAS	0.2 %	0
DDR2	0	2.2 %

Different Signal Transduction Pathways in NSCLC

EGFR Signal Transduction Pathway

Since the therapeutic advantage of growth factor inhibitor had been confirmed for the treatment of breast cancer in Her-2 positive patients, a large number of clinical trials were launched to confirm the clinical effect of EGFR inhibitors in the case of lung cancers. In 2004, the identifications of somatic mutations in the EGFR gene provided the first glimpse of clinically relevant oncogene. EGFR mutations, which are associated with objective responses to single agent TKI therapy in lung adenocarcinoma, are more common in females of Asian ethnicity, who have never smoked and who have adenocarcinoma with lepidic growth pattern. The EGFR mutations are approximately 10–15 % in Caucasians and 20–50 % of all NSCLCs in East-Asians [11]. In adenocarcinomas, the majority of mutations have been identified in exon 18–21 of the gene. These mutations can be classified into three major

categories: in-frame deletion in exon 19, insertion mutation in exon 20, and missense mutations in exon 18–21. The most frequent mutations were located at exon 19 and exon 21. The most commonly used method to detect EGFR mutations is direct sequencing. The EGFR tyrosine kinase modulates cell proliferation and survival through autoactivation of EGFR itself, or through two downstream pathways: the PIK3CA/AKT/MTOR and the RAS/RAF1/MAP2K1/MAPK1 pathways [12]. EGFR mutations are generally associated with sensitivity to TKI therapy. The EGFR mutation is adenocarcinoma specific, but the most EGFR mutations are detected in adenocarcinomas [13]. KRAS has a key role in the EGFR signalling network. Mutations of KRAS are present in 25–35 % of TKI non responsive cases. KRAS mutations are oncogenic missense mutations that develop predominantly in adenocarcinoma histology. KRAS mutations are commonly G→T transversions and occur more frequently in smokers with lung adenocarcinoma. The frequency of KRAS mutations was higher in smokers than in never smokers in the study of 106 patients with lung adenocarcinomas (43 % vs. 0 %; $P=0.001$). However, a more recent study done with 482 lung adenocarcinomas by Riely demonstrated that KRAS mutations occur in about 15 % of the adenocarcinomas of never smokers [14]. KRAS mutations were found in 17 % of African-American patients compared with 26 % of Caucasian patients [15].

A KRAS mutation is a negative predictor of response to anti EGFR monoclonal antibodies and is also an important mechanism of a primary resistance to EGFR-TK inhibitors. KRAS mutational status is already of significant utility in the development and selection of patients for clinical trilas and will become an important biomarker in the treatment of patients with advanced NSCLC [16].

The prognostic and predictive value of KRAS mutations in patients with lung cancer is controversial in the literature. Biases in disease stage, treatment regimen, small-scale patient studies, and biomarker status have led to inconsistent results. In this last study,the KRAS and EGFR genes status were examined in 1935 consecutive patients with NSCLC. All patients were divided into mutation groups (KRAS group, EGFR group) and KRAS/EGFR wild type (WT group) groups. The overall survival was 14.47 months in the KRAS mutant group, 20.57 months in the KRAS WT group, and 42.73 months for the EGFR group, ($P<0.001$). Multivariate analysis indicated that the KRAS mutation status was an independent prognostic factor (hazard ratio 2.69, 95 % confidence interval 1.91–3.8, $P<0.001$). No difference was found in PFS and tumor responsiveness between patients with KRAS mutation and those with wild type KRAS/EGFR for chemotherapy and EGFR tyrosine kinase inhibitors (TKI). PFS did not significantly differ for chemotherapy among the three groups ($P=0.270$). The conclusion that is based on this study is the following: KRAS mutation is a poor prognostic factor; however, it is not an independent predictor of response to EGFR-TKI or chemotherapy in patients with lung cancer [17]. EGFR mutations are the best predictive biomarkers over clinicopathologic features in predicting tumor response and progression free survival to EGFR TKIs. The meta-analysis showed a therapeutic response over 70 % in cases of EGFR mutation of adenocarcinoma (exon 19, exon 21 mutation). Therefore EGFR mutation is a significant predictive factor in cases where EGFR TK inhibitors are used [18].

Agents Targeting the EGFR Pathway

First Generations EGFR TK Inhibitors

Erlotinib (Tarceva®, Roche)

Erlotinib monotherapy is recommended as a first, second and third line treatment in advanced NSCLC patients who have activated EGFR mutations. Adenocarcinoma histology, never smoking history, female gender and East Asian ethnicity were found as a prognostic factors and correlate with the frequency of EGFR mutations and EGFR gene amplification [19]. According to the Phase III SATURN trial, erlotinib maintenance therapy following first line platinum-based chemotherapy significantly increased the PFS compared with placebo. (12.3 weeks versus 11.1 weeks HR: 0.71). PFS benefit was greatest in patients whose tumors had activated somatic mutations in the EGFR gene. When given as maintenance therapy, erlotinib appears to delay progression and prolong survival regardless of clinical or molecular characteristics [20]. On the basis of this, erlotinib was approved in Europe as a monotherapy for maintenance treatment in patients with stable disease after first line platinum-based first line chemotherapy. Determining response to erlotinib plus chemotherapy combinations based on EGFR status is a further challenge. OPTIMAL trial was the first, prospective phase III study to investigate erlotinib versus gemcitabine/carboplatin for the first line treatment of Chinese patients with NSCLC, selected by EGFR mutation status. There was a significant PFS difference between the two study arms to the advantage of erlotinib of 13.1 versus 4.6 months of chemotherapy (HR: 0.16 $p\leq0.0001$). This significant difference was demonstrable in cases of EGFR exon 19 and 21 mutation [21]. The role of erlotinib in wild type EGFR was investigated in the phase III TAILOR trial. There was a comparison between erlotinib and docetaxel as a second line treatment, having overall survival (OS) and progression free survival (PFS) as principal and secondary endpoints. When the PFS results were compared, it was found that there was a superiority of docetaxel over erlotinib as second line treatment for patients without EGFR mutations in exon 19 or 21 [22]. First line setting was registered by the results of OPTIMAL [21] and EURTAC [23] trials. Compared

with standard chemotherapy, erlotinib conferred a significant progression-free survival benefit in patients with advanced EGFR mutation-positive NSCLC and was associated with more favourable tolerability. Erlotinib has shown to improve progression-free survival compared with chemotherapy when given as first-line treatment for Asian patients with non-small-cell lung cancer (NSCLC) with activating EGFR mutations. The study was aimed to assess the safety and efficacy of erlotinib compared with standard chemotherapy for first-line treatment of European patients with advanced EGFR-mutation positive NSCLC. Eligible participants were adults (> 18 years) with NSCLC and EGFR mutations (exon 19 deletion or L858R mutation in exon 21) with no previous history of chemotherapy for metastatic disease. The primary endpoint was progression-free survival (PFS) in the intention-to-treat population. The median PFS was 9.7 months (95 % CI 8.4–12.3) in the erlotinib group, compared with 5.2 months (4.5–5.8) in the standard chemotherapy group (hazard ratio 0.37, 95 % CI 0.25–0.54; $p<0.0001$). Erlotinib reduced the risk of lung cancer progression by 66 % (HR=0.34, $P<0.0001$).

Gefitinib (Iressa®, AstraZeneca)

Gefitinib is also an EGFR-TK inhibitor. It is registered in the Asia–Pacific region as a first line treatment for patients with advanced NSCLC, and it is approved in Europe as a single agent treatment for NSCLC patients with EGFR activating mutations. In the US, gefinitib is approved as a continued monotherapy for patients with EGFR mutation after the failures of primary platinum-based and docetaxel therapies. Approval was based on the pivotal phase III trials in selected patients (INTEREST and IPASS) [23, 24]. In the IPASS trial, gefitinib significantly delayed cancer progression (median PFS 9.5 months vs. 6.3 months; HR 0.48, 95 % CI 0.36 to 0.64, $p<0.0001$) and improved tumour shrinkage for NSCLC patients with the activating mutation of EGFR-TK compared with standard doublet chemotherapy (71.2 % v 47.3 %, $p=0.0001$), while in the INTERESTstudy, gefitinib demonstrated equivalent survival to chemotherapy for patients who progressed following chemotherapy. In both trials gefitinib demonstrated a better tolerability profile and quality of life benefits than chemotherapy. With regard to all the clinical end points of the IPASS trial (PFS, ORR, tolerability, QOL), a more favourable result was obtained in patients with EGFR mutation treated with gefitinib in comparison with carboplatin/paclitaxel in Asia [25]. PFS was significantly longer with mutated EGFR status compared with wild type EGFR tumors suggesting that patients who are mutation negative should be selected for chemotherapy rather than anti-EGFR treatment [26]. In the Phase III INTEREST trial, the second line single agent gefitinib was non-inferior versus docetaxel in terms of survival. Concerning to ORR, PFS and QOL, a significant benefit was demonstrated in patients with EGFR mutations. In elderly patients with advanced EGFR mutated NSCLC the first line gefitinib therapy has also demonstrated efficacy with acceptable and manageable toxicity in phase II study [27].

Cetuximab (Erbitux®, Merck)

Cetuximab is a chimeric-type monoclonal antibody (mAb) that binds to the extracellular domain of EGFR. This class of treatment only inhibits ligand dependent activation of EGFR and not autophosphorylation of the tyrosine kinase domain. These mutations may still activate the downstream pathways, and upregulate cell cycle progression, cell growth and angiogenesi. In the FLEX study chemotherapies with and without cetuximab was compared as first-line treatments in patients with advanced NSCLC, who had tumour expressing even a minimum amount of EGFR (>1 % cells). The study showed better survival for the treatment with chemotherapy plus cetuximab (median survival 11.3 months vs. 10.1 months). There was no recorded corresponding survival benefit for patients in the low EGFR expression group (median 9.8 months vs. 10.3 months $p=0.88$). The Hirsh score (H) takes into account the percentage of cells (0–100 %) in each intensity category (0–3+) and computes a final score, on a continuous scale between 0 and 300. In this study, a cutoff value of 200 was used to define high and low EGFR expressing groups. The H score assessment seems to help select a group of patients that might particularly benefit from EGFR TKIs [28]. Cetuximab used in combination with either gefitinib or erlotinib showed synergistic and antiproliferative activity against head and neck cancer cell lines and lung cancer cell lines that expressed different levels of EGFR. When we combine two reversible inhibitors, if the TKI is replaced by ATP at the cell surface, there is a potential for EGFR reactivation. The co-binding of antibody reduces this activation by preventing ligand induced or ligand independent dimerization, leading the more sustained EGFR inhibition, and this is one of the important ways in which dual targeting causes greater EGFR inhibition [29].

Second Generation EGFR TK Inhibitors

Afatinib (Giotrif ®, Boehringer Ingelheim)

Afatinib is an irreversible EGFR and Her-2 TK inhibitor with a preclinical activity against cells containing the classical resistance mutations as well as EGFR T790M. In the LUX-LUNG Phase II/b III double –blind randomized trial, Best Supportive Care (BSC) plus either afatinib or placebo were conducted in patients with NSCLC who had failed one or two lines of chemotherapy and erlotinib or gefitinib. This study showed no significant OS difference between treatment arms;

however, it was suggested that this may be due to enrichment with patients with EGFR mutations for whom survival times can be as long as 11 months in the third or fourth-line setting. However, there was a significant improvement in median PFS with afatinib versus placebo (3.3 months vs. 1.1 month, the mutation ratio was approximately 50 %) and a significant decreases in tumor related symptoms (dyspnea, pain and cough) [30]. Two phase III trials (LUX-LUNG 3 and LUX –LUNG 6) are currently evaluating afatinib as a first-line treatment setting. The excellent result of LUX –LUNG 3 was presented at 2012 annual ASCO meeting. This is the first study using pemetrexed/cisplatin combination as a comparator. Treatment with afatinib significantly prolonged PFS compared to treatment with pemetrexed/cisplatin. Adverse events with afatinib were manageable. With 4.2 months PFS improvement in the overall population and 6.7 months in patients with mutations, afatinib is become a clinically relevant first line treatment option [31]. Afatinib is approved by FDA at July 2013, and September 2013 by EMA for patients with late stage (metastatic) non-small cell lung cancer (NSCLC) whose tumors express specific types of epidermal growth factor receptor (EGFR) gene mutations. Afatinib may also work well in combination with cetuximab for patients who have become resistant to treatment with erlotinib. A study found that the objective response rate in the first 60 evaluable patients enrolled in a study testing the combination was 30 % [32].

Dacomitinib (PF-0299804, (Pfizer))

Dacomitinib is an orally administered, irreversible pan-HER inhibitor, inhibiting of EGFR/HER1, HER2 and HER4 Tyrosine kinases. Dacomitinib has demonstrated activity against acquired resistance of EGFR to gefitinib or erlotinib [33]. In a phase II study in advanced NSCLC, treatment with PF-0299804 resulted tumor shrinkage in all patients with typical EGFR activating mutations in exon 19 or 21. There was a preliminary evidence of antitumor activity in EGFR wild type (WT) patients and those with mutation in exon 20. Median PFS was 9.63 months, disease control rate was 96 %. In another phase II study, in advanced NSCLC, PF-0299804 across a broad range of molecular subgroups: overall PFS 12 were 4 weeks versus 8.4 weeks (HR 0.704 $p=0.030$) was demonstrated. This trial enrolled a cohort of patients with K-RAS wild type (WT) disease whose PFS also improved with PF-0299804 compared with erlotinib(16. 6 weeks versus 8.4 weeks: HR 0.551, $p=0.004$).

Neratinib (HKI-272, Pfizer)

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have had a significant impact on (NSCLC) outcomes, particularly among patients with *EGFR* mutations. Resistance emerges after 9 to 12 months, primarily mediated by the T790M resistance mutation. Neratinib is an irreversible pan-ErbB TK inhibitor that may overcome T790M. Neratinib has low clinical activity with 240 mg daily dose in NSCLC patients previously benefiting from first-generation EGFR TKIs and among TKI-naïve patients with adenocarcinoma and little or no smoking history. Significant and durable responses were seen among the small cohort of patients with G719X mutations in EGFR. Future studies with neratinib in NSCLC to modify the dose and/or schedule to mitigate diarrhea and allow for achievement of higher biologic doses is needed [34].

Pelitinib (EKB-569, Wyeth/Pfizer)

Pelitinib is a pan-ErbB is a tyrosine kinase inhibitor with potential antineoplastic activity. Pelitinib irreversibly binds covalently to epidermal growth factor receptors (EGFR) ErbB-1, -2 and -4, inhibiting receptor phosphorylation and signal transduction resulting apoptosis and suppression of proliferation of tumor cells that overexpressing these receptors, Phase I trial is ongoing.

Canertinib (CI-1033, Pfizer)

Canertinib binds irreversibly to the intracellular domains of epidermal growth factor receptor tyrosine kinases (ErbB family), inhibiting signal transduction and resulting tumor cell apoptosis and suppression of tumor cell proliferation. This agent also acts as a radiosensitizing agent and displays synergistic activity with other chemotherapeutic agents, Phase II trial is ongoing.

Third Generations EGFR TK Inhibitors These are in early development.

EGFR Mutations and Drug Resistance

Approximately 30 % of patients still do not experience disease responses despite harboring EGFR mutant disease, and less than 5 % experience a complete responses [35]. Most driver mutations present in resistant tumors. Furthermore, EGFR mutations, ALK gene rearrangements, and KRAS mutations rarely coexist in treatment-naive NSCLC tumors. 0Acquired resistance to EGFR TKIs in the metastatic setting is inevitable. The average PFS is 10–16 months. The drug resistance remains a major clinical problem in the daily practice.

The mechanisms of primary and secondary resistance to EGFR TKIs should be separated.

Primary Resitance

1. **De novo resitant EGFR mutations**. Tumors with EGFR exon 20 insertion (presented in 4 %) are associated with a lack of drug sensitivity in preclinical models and in clinical settingss. More than 50 % with acquired resistance to gefitinib or erlotinib is due to T790 mutation (subsitution of methioin for threonine) at position of 790.
2. **Suboptimal drug exposure**. This result in lack of anti-tumor effect. The patients were found to have low plasma concentration of drug.
3. **Failure of apoptosis induction**.
4. **Other potential mechanisms**. Other cell intrinsic factors may affect TKI sensitivity. Approximately 50 % of NSCLCs, especially adenocarcinomas, harbour recurrent somatic alterations in genes that encode components of major signalling pathways: ALK, ROS1, RET, HER2, KRAS, NRAS, PIK3CA, AKT1, BRAF and MEK1. Among these, PIK3CA mutations have been shown to be acquired after patients develop resistance [36]. Hepatocyte growth factor (HGF), the ligand of the MET receptor tyrosine kinase, was found to overexpress in 29 % of primay resistant tumors with drug sensitive EGFR mutation [37].

Secondary Resistance

1. **Second –site EGFR mutations**. This is the most frequent mechanism of acquired resistance to EGFR TKIs in lung cancer, found in more than 50 % of patients. More than 90 % are composed of the T790 gatekeeper mutation.
2. **Suboptimal drug exposure in the brain**. Approximately 33 % of patients with EGFR –mutant lung cancer treated with EGFR TKIs will experience disease progression in the brain. Drug concentration of drug achievable in the brain is 1–5 % of the level found in the plasma. High –dose EGFR TKI has been shown to be clinically tolerable and potentially beneficial without dose-limiting toxicity.
3. **Activation of EGFR signalling pathways with other aberrant molecules**. Overexpession of MET activates the PIK3/AKT pathway via interaction with ERBB3, rendering cells less dependent solely on mutant EGFR for survival.
4. **Histologic transformation**. Rebiopsy of growing tumors has obtained cells that no longer display adenocarcinoma histology. They still harbour a drug sensitivity EGFR mutation, the cells display features of small –cell lung cancer (Table 3).

Table 3 EGFR mutations and drug resistance

Primary resistance	Secundary resistance
1. De novo resistants EGFR mutations	1. Second-site EGFR mutations
2. Suboptimal drug exposure	2. Suboptimal drug exposure int he brain
3. Failure of apoptosis induction	3. Activation of EGFR signalling pathways with other aberrant molecules
4. Other potential mechanisms	4. Histologic transformation

Treatment Possibilities to Overcome Resistance

1. **Second and third generation EGFR TKIs**. Second generation EGFR TKIs include canertinib, neratinib, afatinib and dacomitinib. The third generation EGFR inhibitors include WZ4002 and CO1686.
2. **Drug combinations**. The combination of erlotinib with cetuximab showed no effect in patients who acquired resistance to EGFR TKIs [38].
3. **Tratment beyond progression**. Patients who acquire resistance can respond to EGFR TKIs after drug holiday. Resistant tumors are composed of mixed populations of sensitive and resistant cells and suggest a benefit of continued EGFR TKI administration even after the acquisition of resistance.
4. **Novel combinations**. The most promising strategy administers low –dose continous EGFR TKI in combination with high –dose pulsed doses with the goal of preventing the replication of EGFR TKI-sensitive cells so as to optimally delay the emergence of resistant clones [39].

VEGFR Pathway

VEGF regulates vascular permeability, and is a key player in tumour angiogenesis, acting as an endothelial cell survival factor. Endothelial cell proliferation, migration and survival are mediated by VEGFR-1 and VEGFR-2, and several agents targeting VEGF or one or more of its receptors have been developed. The PDGF pathway is also integral to the initiation and regulation of angiogenesis. Elevated levels of VEGF and PDGF have been linked to tumor progression and decreased survival in NSCLC patients [40].

Bevacizumab (Avastin® Roche)

Bevacizumab is an anti-VEGF monoclonal antibody (mAb) and has demonstrated clinical benefit in numerous tumor types, including NSCLC. While bevacizumab had limited activity as a single agent, clinical benefits were observed in ECOG 4599 trial (paclitaxel/carboplatin plus bevacizumab versus chemotherapy alone). As the result of this trial, it was

registered for the first line treatment of advanced non-squamous NSCLC in both the USA and Europe [41]. Avastin is indicated for the first-line treatment of unresectable, locally advanced, recurrent or metastatic non–squamous non–small cell lung cancer in combination with carboplatin and paclitaxel [42]. However, there is some evidence to suggest that the safety profile of bevacizumab may be acceptable in some of these patient subgroups, including those with brain metastases and those receiving anticoagulant therapy. For patients who are not suitable for bevacizumab, the standard of care continues to be platinum-based doublet. In the AVF0757g study, the incidence of severe or fatal pulmonary hemorrhage was 31 % in patients with squamous histology and 4 % in patients with histology other than squamous cell carcinoma, suggesting that squamous cell histology may be one of the possible risk factors for the development of a severe pulmonary hemorrhage [43].

Axitinib (Inlyta ®, Pfizer)

Axitinib is an oral, potent, selective inhibitor of VEGFRs 1–3 with high potency for VEGFR-2. In a phase II study patients with advanced NSCLC, axitinib demonstrated single-agent activity and treatment was well tolerated. Further investigation of the activity of axitinib both as a single agent and in combination with chemotherapy for advanced NSCLC is underway [44].

Aflibercept (Zaltrap ®,Sanofi-Aventis)

Aflibercept is a protein comprising segment of the extracellular domains of VEGFR-1 and VEGFR-2 that binds VEGF. Aflibercept prevents the binding of VEGF to its receptor, resulting in tumor regression and inhibition of tumor angiogenesis and metastasis [45]. A phase III trial evaluating aflibercept in combination with recurrent or refractory NSCLC is ongoing.

Multikinase Inhibitors (MTKIs)

Cediranib (Recentin®, AZD2171, AstraZeneca)

Cediranib is a VEGFR Tyrosine Kinase inhibitor (TKI), that also inhibits PDGFR at sub-clinical levels. It has shown preliminary activity in patients with advanced NSCLC in phase I study. In a phase II trial, in combination with carboplatin/paclitaxel, the response rate was higher for 30 mg cediranib than placebo (38 % versus 16 %) but toxicity was increased, and 37 % of patients required dose modification to 20 mg [46].

Vandetanib (Zactima® ZD6474, AstraZeneca)

Vandetanib is a multikinase inhibitor of VEGFR and EGFR and RET, but with pharmacologically achievable doses it is a more potent inhibitor of VEGFR than EGFR [47]. Based on promising phase II data, four randomised phase III trials were conducted to investigate the efficacy of vandetanib in refractory patients with advanced NSCLC. ZODIAC and ZEAL studies investigated vandetanib in combination with chemotherapy, ZEPHYR l compared vandetanib with Best Supportive Care and ZEST trial compared vandetanib with erlotinib. Only ZODIAC trial met its primary endpoint. Median PFS was 4 months in the vandetanib group, compared with 3.2 months in the placebo group. In 2009, AstraZeneca withdrew a regulatory application for the use of vandetanib in combination with chemotherapy in patients with NSCLC.

Sunitinib (Sutent®, Pfizer)

Sunitinib is an oral multi-target Tyrosine Kinase inhibitor (MTKI). It inhibits VEGFRs, PDGFRs and the stem- cell factor receptor. Dual inhibition of VEGFR and PDGFR with sunitinib resulted in antitumor activity in preclinical investigations. When single-agent sunitinib was given by either intermittent or continous daily dosing, it was shown to have activity and to be tolerable in an open-label phase II study of patients with advanced, platinum refractory NSCLC [48]. Sunitinib was demonstrated to have potential as maintenance therapy in Phase II trial in patients with advanced NSCLC, and a phase III maintenance trial conducted by the NCT00693992 is underway. Data from clinical trials indicate that sunitinib combined with docetaxel, gemcitabin and pemetrexed have manageable safety profiles and preliminary evidence of antitumor activity in patients with advanced solid tumors, including NSCLC.

Sorafenib (Nexavar®, Bayer)

Sorafenib is an oral multikinase inhibitor (MTKI) with activity against C-RAF and B-RRAF kinase, VEGFR-2 and VEGFR -3, PDGFR and C-Kit. Sorafenib may have a dual mechanism of action targeted directly the tumor and tumor angiogenesis by inhibiting MEK and ERK phosphorylation in cancer cell. In phase II studies single –agent sorafenib prolonged PFS and stabilized disease in patients with relapsed or refractory advanced NSCLC [49].

Motesanib (AMG706, Amgen)

Motesanib is an oral multikinase inhibitor (MTKI), which targets VEGFRs, PDGFRs, KIT and RET. Motesanib inhibits VEGF-induced cellular proliferation and vascular permeability [50]. A phase II study of chemotherapy plus two dosing

schedules of motesanib versus chemotherapy plus bevacizumab revealed similar efficacy in both arms, the arm with continous daily regimen of motesanib was performing marginally better than the intermittent schedule – (median PFS 8.4 months compared with 6.2 months)[51]. Enrollment of patients with squamous –cell histology into the phase III MONET trial evaluating motesanib with carboplatin/paclitaxel for the first line treatments of advanced NSCLC was suspended due to concerns over early mortality rates. This MONET trial has been reopened and patients with non-squamous histology are to be enrolled.

Ramucirumab (IMC-1121B, Lilly)

Ramucirumab is an investigational monoclonal antibody that binds to VEGFR-2 and blocks ligand binding and activation. A phase II open-label study is currently evaluating ramucirumab as first-line NSCLC therapy in combination with carboplatin/paclitaxel, with preliminary results from the first 15 patients reporting an overall RR of 67 %. Another phase II trial is recruiting patients with previously untreated NSCLC to examine ramucirumab in combination with four different chemotherapeutic regimens as first-line therapy (NCT01160744), and a phase III trial is recruiting patients with NSCLC to test ramucirumab in combination with docetaxel as second-line therapy after failure of platinum based therapy (NCT01168973) [52].

Nintedanib (Vargatef®, BIBF 1120, Boehringer Ingelheim)

Nintedanib inhibits VEGFR-1, -2 and -3, PDGFR-α/β, and FGFR-1, -2 and -3, and also has activity against members of the v-src sarcoma viral oncogene homolog (Src) family and fms-like tyrosine kinase 3 (flt-3). In a phase I trial in patients with NSCLC, when nintedanib was combined with pemetrexed, SD was achieved in 13 of 26 patients (50 %). The LUME-Lung 1 phase III trial (NCT00805194) is currently evaluating nintedanib plus docetaxel vs. placebo plus docetaxel as second-line therapy for NSCLC, with an estimated enrollment of 1,300 patients and the primary endpoint is PFS. The LUME-Lung 2 phase III trial (NCT00806819) has also been initiated to evaluate PFS with nintedanib plus pemetrexed vs. placebo plus pemetrexed as second-line NSCLC treatment, with an estimated enrollment of 1302 patients [52].

Pazopanib (Votrient ®, GW786034; GlaxoSmithKline)

Pazopanib inhibits VEGFR, FGFR, PDGFR and c-kit. A phase II trial ($N=35$) evaluated pazopanib monotherapy in patients with resectable NSCLC, andthe primary endpoint was RR. AEs were generally grade≤2 and included hypertension (43 %), diarrhea (37 %), fatigue (37 %) and nausea (34 %). Only 8.6 % PR was achieved. Pazopanib is investigated with pemetrexed in patients with stage IV NSCLC who did not progress (SD, PR or complete response) after induction therapy containing platinum and pemetrexed [52].

Linifanib (ABT-869, Abbott)

Linifanib inhibits all three VEGFR isoforms, and PDGFR. In a randomized phase II trial 139 patients with advanced NSCLC progressing after previous therapy were involved. The most common grade 3/4 linifanib-related event was hypertension (14 %). A phase II trial (NCT00716534) is ongoing to test two doses of linifanib with carboplatin/paclitaxel as a first-line treatment for patients with advanced non-squamous NSCLC [45].

Other Signal Transduction Pathways

EML-4 ALK Rearrangement: Driver Mutation of Lung Cancer

A special ALK gene rearrangement such as the aberrant EML4-ALK fusion gene has recently been described in NSCLC. The resulting cytoplasmic chimeric protein has constitutive kinase activity in the case of NSCLC. The most common fusion results from exons 1–13 of EML4 joining exons 20–29 of ALK. At least seven EML4- ALK variants have been identified in lung adenocarcinoma. As a result of this rearrangement, it has kinase activity and located in the cytoplasm [53]. These gene rearrangements exist approximately 4-6 % of all lung cancers, seem to be mutually exclusive with activating EGFR or KRAS mutations and are more frequent in adenocarcinoma and non-smokers and younger people. This gene rearrangement was first detected in anaplastic large cell lymphoma, neuroblastoma and myofibroblastic tumors [54].

MET Amplification

MET also contributes to primary and acquired resistance to EGFR TKIs. MET is a proto-oncogene located on 7q21 chromosome, which encodes the tyrosine kinase, hepatocyte growth factor receptor (HGFR). The hepatocyte growth factor receptor protein possesses tyrosine –kinase acitity. The amplification of MET is associated with acquired resistance to therapy with an EGFR TKIs. MET amplification has been reported in about 20 % of tumors among patients with acquired resistance. MET amplification occurs in both adenocarcinoma and squamous cell carcinoma. The amplification of MET is correlated with poor prognosis [55].

Onartuzumab®(MetMab, Roche)

Onartuzumab is the MET Mab, a monovalent monoclonal antibody which specifically binds to the MET receptor, blocking activation mediated by the hepatocyte growth factor (HGF). Dual inhibition of MET and EGFR has also been investigated in models of EGFR –resistant NSCLC. Because of the results of Phase II clinical trials, Phase III study is ongoing.

ROS1 Rearrangements

ROS1 is a receptor tyrosine kinase of the insulin receptor family and regulate the MAPK signalling cascade as an oncogene in NSCLC. Chromosomal rearrangements involving the ROS1 gene were originally described in glioblastomas. Rearrangements were found to be more common in never smokers and Asian patients and were associated with younger age, and with adenocarcinoma. It has been identified that cells carrying ROS1 rearrangements have sensitivity to ALK inhibitors. After confirming that this cell line was similarly sensitive to crizotinib, investigators treated patients with ROS1 rearranged lung adenocarcinoma with crizotinib, and has a near complete response [56].

RET Rearrangements

These rearrangements were recently identified in a subset of lung adenocarcinomas, from never smokers or former light smokers, who were wild type for: EGFR, KRAS, ALK, HER2, BRAF and ROS1 oncogenic alterations. These RET agents approved for medullary thyroid cancer.

Activated AKL Tyrosine Kinase and C-MET and ROS1 Targets

Crizotinib (Xalkori ®, PF 02341066, Pfizer)

Crizotinib is a novel oral selective ATP-competitive inhibitor of ALK and c-MET tyrosine kinase that inhibits tyrosine kinases. Crizotinib inhibits tyrosine phosphorylation of activated ALK at nanomolar concentrations [57]. The new fusion oncogene was identified in 2007 by Soda et al. Approximately 2-7 % of patients with NSCLC have tumor with inversion in the short arm of chromosome 2 that results in the fusion of the echinoderm microtubule-associated protein like 4 (EML-4) gene with the ALK gene leading to the production of an EML-4 ALK fusion tyrosine kinase. ALK is a transmembrane protein, which has a kinase domain and is not usually expressed in the lung. [53] In cell line and mouse models, EML-4-ALK is highly oncogenic, activates the PI3K-AKT and MAPK-ERK pathways and induces lung tumors. Specific clinical features of tumor associated with ALK translocations include never or light smoking history, young age, and adenocarcinoma histology with signet rings. ALK translocations are usually mutually exclusive with EGFR or K-RAS mutations and predict poor response to EGFR TKIs in patients with advanced NSCLC. The methods of detection of ALK translocations in NSCLC have not been well standardized. Detection methods are immunohistochemistry (IHC), fluorescence in situ hybridisation (FISH), or with reverse transcriptase technique [58]. In preclinical studies specific ALK inhibitors have demonstrated activity against tumors with ALK translocations. The most advanced small-molecule TKI targeting ALK translocations in NSCLC is crizotinib (PF 02341066) which was developed as a MET inhibitor, but also has a functions as an inhibitor of ALK [59]. Results from the initial phase I clinical trial of crizotinib and the expansion cohort of the recommended phase II dose for ALK translocated NSCLC have been recently presented and subsequently published [60]. Approximately 1500 NSCLCs were screened by the ALK break –apart FISH probe, and 82 patients were enrolled. Patients were treated with 250 mg of crizotinib orally twice a day. The confirmed RR was 57 %, with an additional 33 % of patients who met the criteria for stable disease (SD), including 6 % who had unconfirmed partial responses (PR). Median PFS has not yet been reached; however, it is estimated 72 % with a medial follow-up at 6 months. The most common reported toxicities included nausea, vomiting, diarrhea, mild visual disturbances, liver function test abnormalities, and peripheral edema [61]. On the basis of this excellent result, 3 years after human tests the Phase III registration trial were initiated. The result of the crizotinib Phase III trial, crizotinib was registered by the FDA for the treatment of advanced ALK positive NSCLC on 26 August 2011.

LDK378 (Novartis)

Non-small-cell lung cancer with ALK rearrangements is sensitive to the tyrosine kinase inhibitor crizotinib (Xalkori®) but invariably develop resistance. LDK378 is a novel, more potent ALK tyrosine kinase inhibitor than crizotinib with significant antitumor activity in preclinical models. In a Phase I multicenter study, 131 patients with genetic alteration in ALK were enrolled. LDK378 was administered orally at doses of 50–70 mg once a day. LDK378 induces durable responses in the majority of the patients with advanced ALK positive NSCLC [62].

Tivantinib® (ARQ 197, Daiichi Sankyo)

ARQ 197 is an orally available, selective inhibitor of the c-MET receptor tyrosine kinase. Preclinical data have demonstrated that ARQ 197 inhibits c-MET activation and shows antitumor activity against several human tumor xenografts. In a phase II trial in patients with advanced refractory NSCLC,

ARQ 197 in combination with erlotinib prolonged PFS 16.1 weeks versus 9.7 weeks compared with erlotinib and placebo, but this was not statistically significant. PFS benefit observed with ARQ 197 was promising among patients with non-squamous histology, EGFR wild type status and KRAS mutations. Time to distant metastasis was significantly improved with ARQ 197 plus erlotinib versus placebo plus erlotinib in patients with advanced NSCLC [63].

Cabozantinib (Cometriq, XL184,BMS 907351)

Cabozantinib is a MET, EGFR, VEGFR-2 and RET inhibitor. Cabozantinib was assessed in combination with erlotinib in a phase IB/II trial among 54 patients with NSCLC, most of whom had received previous erlotinib treatment. This study suggests a role for MET in acquired resistance to EGFR inhibitors and demonstrates that combined inhibition of EGFR and MET can overcome resistance to EGFR inhibitors [64].

Combining targeted agents is a recent approach to the treatment of advanced NSCLC. Combining agents with complementary mechanism of actions may inhibit signalling pathways more completely, but may compromise the safety profile of the regimen. Therefore, each combination needs to be evaluated carefully.

VEGFR+EGFR Combined Inhibition

When using NSCLC target therapy with the modification of the signal transduction pathways, VEGFR and EGFR are key targets in NSCLC that share common downstream signalling pathways to influence the tumor and its vasculature. Clinical data of erlotinib and bevacizumab have been encouraging, suggesting an additive benefit for patients with advanced NSCLC [65]. In the Phase III/B study, erlotinib+placebo or erlotinib+bevacizumab combinations were administered. The study failed and did not meet its primary endpoint of improving OS in second and third line setting (9.3 months versus 9.2 months for erlotinib and placebo) [66]. In the ATLAS Phase III multicenter, randomized, placebo-controlled trial, bevacizumab and erlotinib as a maintenance therapy in patients with locally advanced or metastatic NSCLC was terminated early at ithe second interim analysis because it met the primary endpoint of PFS. There appeared to be an OS benefit (15.9 months with bevacizumab plus erlotinib versus 13.9 months with bevacizumab alone), but the study was not powered to detect an OS difference [67]. The addition of cetuximab to bevacizumab and paclitaxel/carboplatin was investigated in randomised phase II study in which acceptable toxicity was observed. In another study, bevacizumab was added to paclitaxel/carboplatin/cetuximab and followed by bevacizumab and cetuximab as maintenance therapy. This combination also demonstrated an efficacy and acceptable safety and tolerability profile [68]. The combined cost of two targeted agents plus chemotherapy will have to be balanced carefully against potential clinical benefit.

VEGFR+PDGFR+EGFR Inhibition

Targeting PDGFR in addiction to VEGFR and EGFR may further increase the potential antitumor activity. A recent single –arm, phase II study evaluating erlotinib and sorafenib as first line treatment in patients with NSCLC demonstrated that this regimen was feasible, and patients with adenocarcinoma benefited the most [69]. The combination of erlotinib and sorafenib was also shown to be feasible in elderly patients with advanced NSCLC and was associated with higher 1 year survival rate compared with sorafenib plus gemcitabine. Sunitinib plus erlotinib is a VEGFR+PDGFR+EGFR inhibition treatment strategy that may confer additive or synergistic antitumor effects and may potentially increase clinical benefit in patients with advanced NSCLC. Preclinical data suggest that sunitinib and erlotinib given together are associated with greater antitumor activity compared with each individual agent given alone. Early data from the SUN 1058 study support the administration of sunitinib and erlotinib as a novel VEGFR+PDGFR+EGFR treatment strategy in patient with previously pretreated advanced NSCLC. The primary endpoint was PFS, but the result was not statistically significant (12.3 weeks versus 8.5 weeks), possibly because of low number of events. SUN 1087 failed to reach the primary endpoint of OS, but a significant improvement was observed in the sunitinib plus erlotinib arm for PFS and ORR.

Immunmodulators or Immunotherapies

Subjects with lung cancer were shown to present a variety of immune abnormalities including cellular immune dysfunction, cytokine alterations, and antigen presentation defects. Several immunomodulating agents have activity in this regard including ipilimumab, a monoclonal antibody against the CTLA-4, and talactoferrin, a dendritic cell activator. The anti –PD1 and anti-PD-L1 antibodies potentiate immune responses by blocking the interaction between the PD-1 protein, a T-cell co-inhibitory receptor [70]. In addition, significant activity was shown with belagenpumatucel-L, a whole-cell-based vaccine that blocks the action of TGF-β2. Other promising vaccines are protein-specific vaccines against tumor antigens such as MAGE-A3, EGF, and MUC1. Although some of these immunotherapies may have low performance as single agents in advanced disease, more impressive results are seen in combination with chemotherapy agents [71].

Other Mutations

PIK3CA

Phosphatidylinositol 3-phosphate is a key moderator between the growth factor and the intracellular signal transduction pathway. It is rare in NSCLC, PIK3CA amplification can be demonstrated in greater numbers in male smokers and squamous carcinoma (5 %) and less than 3 % in adenocarcinoma [72]. Ongoing trials investigate in lung cancer single agent PK3K inhibitors as well as combinations with chemotherapy and other targeted agents.

AKT Mutation

The frequency of the AKT1 mutation in cases of NSCLC is 1 %, mainly observed in the US and European population, in cases of epithelial cell carcinoma [73].

BRAF Mutation

BRAF mutation belongs to the MAP kinase signalling cascade and controls cell proliferation. The BRAF mutation can be demonstrated in 1–3 % of adenocarcinoma patients [74]. This mutation is more common in never smokers. BRAF mutant lung adenocarcinoma may be highly sensitive to TKIs like Vemurafenib (Zelboraf®, Roche).

MAP2K1 Mutation

Somatic mutation in MAP2K1 can be detected in 1 % of adenocarcinoma NSCLC patients. With the presence of this mutation, the EGFR, K-RAS, Her-2, PIK3CA and B-RAF mutations are mutually excluded [75].

mTOR (Mammalian Target of Rapamycin) Inhibitors

The mammalian target of rapamycin (mTOR), a serine/threonine kinase, is a downstream mediator in the phosphatidylinositol 3-kinase/AKT signalling pathway, which plays a critical role in regulating basic cellular functions including cellular growth and proliferation. The mTOR activity can be related to the loss of the tumor suppressor gene PTEN and the activation of AKT. It seems that AKT activates mTOR through direct phosphorylation and inhibition of TSC2.

Rapamun ®(Rapamycin, Sirolimus, Pfizer)

Rapamycin is a natural antibiotic, a macrocyclic lactone, which is produced by Streptomyces hygroscopicus, a soil bacterium native to Easter Island (Rapa Nui). Rapamycin, developed initially as an antifungal drug, also possesses immunosuppressive and antiproliferative properties. Inhibition of mTOR by rapamycin also potently inhibits angiogenesis and endothelial cell proliferation in vitro and in vivo. Rapamycin was efficacious in inhibiting the growth of the human NSCLC cells, and in animal models, it effectively inhibited the growth of an NSCLC tumor and alveolar epithelial neoplasia induced by RAS. Evidence that the combination of rapamycin and docetaxel is synergistic in inhibiting the growth of lung cancer led to the hypothesis that mTOR inhibitors could be more efficacious when combined with

Table 4 Summary of oncogenes targets and potential inhibitors

Oncogenes targets	Prevalence (%)	Potential inhibitors
EGFR	5–15	1st. generations:
		Erlotinib
		Gefitinib
		Cetuximab
		2nd. generations:
		Afatinib
		Dacomitinib
		Neratinib
		Pelitinib
		Canertinib
		3rd. generations:
		CO-1686
		WZ4002
VEGFR		Bevacizumab
		Axitinib
		Aflibercept
Multikinase Inhibitors (MTKIs)	1.2–1.9	Cediranib
VEGFR, PDGFR, RET, c-KIT		Vandetanib
		Sunitinib
		Sorafenib
		Motesanib
		Ramucirumab
		Nintedanib
		Pazopanib
		Linifanib
		Crizotinib
ALK, c-MET, ROS1	2.6–7	LDK378
		Tivantinib
		Cabozantinib
		Onartuzumab
Other mutations:		
PIK3CA	<5	GDC-0941
BRAF	<5	Vemurafenib
MAPK2K1	<5	
mTOR		Rapamun
		Afinitor

other agents or therapies, such as chemotherapy or other targeted agents, in lung cancer treatment. No clinical data concerning rapamycin for the treatment of NSCLC are available yet.

Afinitor® (RAD001,Everolimus, Novartis)

RAD001 is an orally available rapamycin analogue showing, in preclinical studies, antitumor effects in cancer cell lines and xenograft models including melanoma and lung, pancreatic, and colon cancer. MTOR inhibitors appear to be well tolerated, with some evidence suggesting antitumor activity. The most common toxicities seen are skin reactions, stomatitis, myelosuppression, and metabolic abnormalities. These adverse events are transient and reversible with interruption of dosing. A series of studies are planned to contribute to the understanding of the role of mTOR inhibitors in NSCLC treatment with regard to the optimal dose, schedule, patient selection, and combination strategies [76]. In the last study, acquired gefitinib-resistant cell lines, together with EGFR wild-type and mutant primary gefitinib-resistant NSCLC cell lines, were treated with everolimus alone, gefitinib alone, or the combination of the two drugs. The combination of everolimus and gefitinib exhibits dose-dependent synergism in primary and acquired gefitinib-resistant NSCLC cells. Thus, a preclinical rationale exists for the use of everolimus to enhance the efficacy of gefitinib in EGFR-TKI-resistant patients with NSCLC [77] (Table 4).

Summary

A huge progress has been made in the field of oncogenic pathway identification in lung adenocarcinoma in the last decades. Targetable pathways are involved in NSCLC and the selected treatment options can work synergistically. However, the use of targeted combination regimens can be optimized, further insights into biology of lung cancer are required. Multiple signalling pathways influence tumor cell survival and proliferation and drug resistance can result escape of tumors. This may be due to of tumor heterogenity and primary and required resistance of lung adenocarcinoma. It can explain why lung cancer is difficult to treat with some targeted therapies, as it is demonstrated by the disappointing results from phase II/III studies involving drugs which showed promise in early clinical trials. Vandetanib, sorafenib and cediranib in lung cancer trials were all stopped early due to futility or toxicity issues, and phase III trials of erlotinib and bevacizumab or erlotinib and sunitinib improved PFS but did not reach their primary endpoints of improving OS. Explorations of newer therapeutic targets such as ALK, c-METand ROS1 as well as co-inhibition of multiple targets such as VEGFR+EGFR or VEGFR+PDGFR+EGFR have demonstrated activity in lung adenocarcinoma. Identifying patients who will most likely benefit from the appropriate treatment is crucial and requires future clinical development. It should include the identification of predictive markers which may enable treatments to be targeted at specific patients groups whereby it can be translated into improved outcomes. The era of personalised medicine when screening becomes a standard precursor to any individual patient's chosen treatment regimen, is within reach for advanced lung cancer [78].

More recent therapy targets, such as the EML-4 ALK, c-MET and m-TOR when used together with other already known targets, may lead to the development of new therapy combinations and protocols. Following this, one of the greatest problems is the identification of patients who will profit the most from the appropriate target therapy. The research on the predictive markers is of key importance. In this way, personally tailored remedies will hopefully become feasible, when molecular screening becomes standard and determinative for the choice of therapy. Better recognition of the molecular biological background of lung cancers, therapy has become much more complex, but at the same time much more efficient. Research on target drugs for NSCLC has resulted in a new way of looking at things. "In accordance with the present trials, we know more predictive biomarkers in the case of lung cancer than in the case of breast cancer, and we have more potential targets available" said Paul Bunn [79]. This is why the "Lung Cancer Mutation Consortium" has been established in the USA, where histological samples from 1000 patients are being collected for more precise definition. Alongside an increasingly broad range of therapy options, the rate of resistance is also growing. Acquired resistance develops after one/one and half year of target therapy.

As the recognition of the molecular factors is being expanded, the options for lung cancer treatment continue to diverge, which contributes to the following goal, which is a precision of the predictive and prognostic markers, in order that personally tailored, targeted and efficient treatments can be accomplished in reality.

It is foreseeable that most patients with NSCLC will have their specific therapy delineated by tumor genotyping in the near future.

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