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Title: Molecular Mechanisms and Targeted Therapies Including Immunotherapy for Non-Small Cell Lung Cancer

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Abstract: Lung cancer is the leading cause of cancer death worldwide. Molecular targeted therapy has greatly advanced the field of treatment for non-small cell lung cancer (NSCLC), which accounts for the majority of lung cancers. Indeed, gefitinib, which was the first molecular targeted therapeutic agent, has actually doubled the survival time of NSCLC patients. Vigorous efforts of clinicians and researchers have revealed that lung cancer develops through the activating mutations of many driver genes including the epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), c-ros oncogene 1 (*ROS1*), v-Raf murine sarcoma viral oncogene homolog B (*BRAF*), and rearranged during transfection (*RET*) genes. Although ALK, ROS1, and RET are rare genetic abnormalities, corresponding tyrosine kinase inhibitors (TKIs) can exert dramatic therapeutic effects. In addition to anticancer drugs targeting driver genes, bevacizumab specifically binds to human vascular endothelial growth factor (VEGF) and blocks the VEGF signaling pathway. The VEGF signal blockade suppresses angiogenesis in tumor tissues and inhibits tumor growth. In this review, we also explore immunotherapy, which is a promising new NSCLC treatment approach. In general, antitumor immune responses are suppressed in cancer patients, and cancer cells escape from the immune surveillance mechanism. Immune checkpoint inhibitors (ICIs) are antibodies that target the primary escape mechanisms, immune checkpoints. Patients who respond to ICIs are reported to experience long-lasting therapeutic effects. A wide range of clinical approaches, including combination therapy involving chemotherapy or radiation plus adjuvant therapy, are being developed.

Keywords: non-small cell lung cancer, EGFR, ALK, ROS-1, BRAF, RET, VEGF, and immune checkpoint inhibitor

1. INTRODUCTION

Non-small cell lung cancer (NSCLC) has been hypothesized to develop based on multistep carcinogenesis induced by chemical and physical mutagens including cigarettes [1], but it has been made clear that NSCLC can develop after even a single gene abnormality[2, 3]. Molecular targeted therapy that blocks the growth and spread of cancer by interfering with molecular targets for NSCLC targets molecular aberrations induced by these gene abnormalities and suppresses cancer cell proliferation and metastasis. Molecular targeted therapy differs from traditional anticancer agents that act on all rapidly dividing cells including normal cells and cancer cells, and includes hormone therapies, signal transduction inhibitors, gene expression modulators, apoptosis inducers, angiogenesis inhibitors, immunotherapies, and toxin delivery molecules. We summarized molecular targeted therapies which dealt with in this review, their associated targets, and acquired mutations conferring resistance in Table 1.

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Gefitinib has been used since 2002, and the prognosis for lung cancer patients has improved year by year[4]. Gefitinib is one of the molecular targeted therapeutic agents targeting epidermal growth factor receptor (EGFR). It was revealed that EGFR-tyrosine kinase inhibitors (TKIs) exert a therapeutic effect specifically for NSCLC with an activated *EGFR* mutation[5]. In addition to gefitinib, EGFR-TKIs include the first-generation EGFR-TKI erlotinib and the second-generation EGFR-TKIs dacomitinib and afatinib, which are irreversible inhibitors of EGFR[6, 7]. Osimertinib, which is a third-generation EGFR-TKI, binds selectively and irreversibly to the activated *EGFR* mutation[8, 9].Oncogenic fusion genes including those involving anaplastic lymphoma kinase (*ALK*) and c-cos oncogene 1 (*ROS-1*), which are sensitive to crizotinib, as well as the rearranged during transfection (*RET*) gene, which is sensitive to vandetanib, were also found to be effective targets for molecular therapy in NSCLC[10-12]. For the treatment of patients with a v-Raf murine sarcoma viral oncogene homolog B (*BRAF*) mutation, dabrafenib or dabrafenib plus trametinib have been used[13]. Although only 5% of NSCLC patients have an *ALK* fusion gene, ALK-TKIs such as crizotinib, alectinib, ceritinib, brigatinib, and lorlatinib are promising anticancer drugs and have contributed to the prominent prognostic improvement[14-16].

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In general, the amount of tumor vessels and the production of angiogenic factors by tumors are related to the malignancy of the cancer in various types of cancer[17-19]. It is thought that tumor blood vessels develop from existing blood vessels using endothelial progenitor cells[20]. The vascular endothelial growth factor (VEGF) pathway plays an important role in the molecular mechanism of this angiogenesis. Bevacizumab is the first anti-VEGF antibody, and it has been successfully used in combination with other anticancer drugs[21]. Ramucirumab is also an angiogenesis inhibitor; it is used in combination with docetaxel and is one of the options after first-line treatment[22]. The combination of bevacizumab and chemotherapy in primary treatment significantly prolonged survival time compared to chemotherapy alone, and second-line treatment with ramucirumab in combination with chemotherapy also significantly prolonged survival time compared to chemotherapy alone[21, 22]. Thus, bevacizumab and ramucirumab enhance the effects of chemotherapy.

Cancer cells with low immunogenicity, which are more difficult for the immune system to eliminate, develop and proliferate by using the immune checkpoint mechanism to negatively control the immune response. Immune checkpoint inhibitors (ICIs) were eventually developed and immunotherapy finally became a standard treatment for NSCLC. Programmed cell death 1 (PD-1) is expressed in immune cells such as T cells and suppresses autoimmunity in the periphery (immune tolerance). PD-1 binds to ligands of antigen-presenting cells (APCs) such as programmed death-ligand 1 (PD-L1) (B7-H1) and PD-L2 (B7-DC) and regulates excessive cytotoxic T lymphocyte (CTL) activity. The anti-PD-1 antibodies nivolumab and pembrolizumab bind to PD-1 on the T cell surface, and the anti-PD-L1 antibodies atezolizumab and durvalumab bind to PD-L1 on tumor cells and tumor-infiltrating immune cells. This leads to enhancement of T cell activity, resulting in antitumor immunity[23]. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is induced by T cell activation and transmits a negative signal by binding to its ligands CD80 (B7-1) or CD86 (B7-2)[24, 25]. Ipilimumab, which is an anti-CTLA-4 antibody, is one of the ICIs, which was developed in combination with other drugs.

Several outstanding articles have been reviewed the molecular targeted for NSCLC[26-29]. In this article, we will highlight the biologic mechanisms and review the approved drugs and currently investigated clinically relevant agents based on MEDLINE and the latest academic information (up to the American Society of Clinical Oncology, ASCO 2018 annual meeting) to help understanding of both clinicians and biologists.

2. MOLECULAR THERAPY TARGETED AT MUTATED CANCER DRIVER GENES

Gene mutations of *EGFR*, *ALK*, *ROS1*, *BRAF*, and *RET* are genetic mutations that directly cause cancer development, and these genes are collectively referred to as driver genes. Patients with these driver genes are reported to have good prognosis after treatment with TKI targeting each gene. The first molecular targeted therapeutic agent against a driver gene was gefitinib, which is classified as a first-generation EGFR-TKI. Erlotinib has a similar function. Gefitinib originally appeared as a drug that inhibits proteins related to cancer proliferation. However, in 2004, an *EGFR* mutation was discovered, and it was found that gefitinib is highly effective for lung cancer patients with an *EGFR* mutation[30]. In a clinical trial of patients with NSCLC harboring an *EGFR* mutation, gefitinib showed a significant PFS prolongation effect compared to chemotherapy[31]. Afatinib, a second-generation EGFR-TKI that had improved effects, was then developed. In 2007, the *ALK* gene was discovered as a second lung cancer driver gene[32]. Translocation in the *ALK* gene leads to carcinogenesis and is frequently found in young people and nonsmokers. Clinical trials reported that the ALK-TKI crizotinib significantly improves PFS for lung cancer patients with an *ALK* gene translocation compared to chemotherapy[10]. It became clear that crizotinib is also effective against lung cancer harboring a *ROS1* mutation, which is one of the driver genes[33]. Alectinib and ceritinib are more effective next-generation ALK-TKIs. Molecular targeted drugs against the newly discovered driver genes such as *BRAF* and *RET* have also been developed. These genetic abnormalities are rare and the establishment of treatment for lung cancers with rare mutations is a major task. A prospective observational study was performed in 733 patients, and 10 driver genes and other genes were found to be mutated in 466 of the patients (64%). The median survival time (MST) of 260 patients who received molecular targeted therapy was 3.5 years, whereas the MST of the patients who did not receive molecular targeted therapy was only 2.4 years (propensity score-adjusted HR, 0.69; 95% CI, 0.53 to 0.9; P=0.006)[34].

Resistance to EGFR-TKIs and ALK-TKIs develops within about 1 year of first administration and the effect of the drugs decrease. One of the resistance mechanisms is thought to involve secondary mutation in *EGFR* or *ALK* that changes the binding site of the drug. There are various types of *EGFR* mutations, among which the T790M mutation is a major cause of first-generation EGFR-TKI resistance. However, even if tolerance develops, treatment with a novel EGFR-TKI is promising, because the proliferation of cancer cells still depends on EGFR. A trial comparing osimertinib and chemotherapy (AURA 3 trial) was conducted on NSCLC patients with the *EGFR* T790M mutation, and the prominent PFS prolonging effect of osimertinib was shown[35]. ALK-TKI resistance is characterized by many types of secondary mutations, and the sensitivity of NSCLC patients to individual ALK-TKIs varies depending on the type of secondary mutation. Crizotinib is effective for NSCLC involving the echinoderm microtubule-associated protein-like 4 (*EML4*)-*ALK* fusion gene[10], but it induces the development of the secondary mutations L1196M and C1156Y[36]. In addition to being sensitive to NSCLC involving the *EML4-ALK* translocation, ceritinib and alectinib as well as brigatinib and lorlatinib are also sensitive to NSCLC with these secondary mutations[37]. We have to pay attention to TKI treatment sequencing. Osimertinib was significantly better than gefitinib and erlotinib in a clinical trial comparing first- and new-generation EGFR-TKIs[9]. Similarly, alectinib was significantly better than crizotinib in clinical trials comparing first- and new-generation ALK-TKIs[14, 38]. However, because no treatment has been established for cancer that has progressed after primary treatment with a new-generation TKI, treatment sequencing should be examined in the future.

2.1. EGFR-TKI

EGFR is a member of the human epidermal growth factor receptor (HER) family, which consists of four molecules: EGFR/HER1/erbB1, HER2/neu/erbB2, HER3/erbB3, and HER4/erbB4[39, 40]. EGFR-TKI competitively inhibits the ATP-binding site in the EGFR tyrosine kinase domain, suppresses auto-phosphorylation of EGFR, blocks downstream signaling, and exerts an antitumor effect[41]. The first-generation EGFR-TKIs (including gefitinib and erlotinib) specifically and reversibly inhibit EGFR, whereas the second-generation EGFR-TKI afatinib irreversibly inhibits EGFR, HER2, and HER4[42]. When a ligand binds to the extracellular domain of EGFR, homodimers are formed between the same molecules, or heterodimers are formed with other HER family molecules. Thereafter, tyrosine residues in the intracellular domain are phosphorylated and various adapter proteins specifically bind to this phosphorylated site. Signals are transmitted to downstream pathways including the RAS-mitogen-activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K)-AKT, and signal transducer and activator of transcription (STAT) pathways, and cell growth, angiogenesis, and metastasis are then induced (Figure 1)[39, 40]. Mutations in the *EGFR* gene have been reported to predict the effect of EGFR-TKIs in NSCLC[5, 30, 43]. These mutations mostly occur in exons 18–21, which code for the intracellular domain of the tyrosine kinase. The most common activating mutations of *EGFR* (>90%) are the in-frame deletion of exon 19 and the L858R missense mutation of exon 21[44]. It has also been reported that *EGFR* mutations correlate with clinical factors that correspond to high susceptibility to EGFR-TKIs such as being female, a non-smoker, or Asian, and having adenocarcinoma[30, 45, 46]. When an EGFR-TKI is administered to NSCLC patients with an activated *EGFR* mutation as a first-line treatment, resistance to EGFR-TKI occurs in most patients, and about 60% of this resistance involves the *EGFR* T790M mutation[47, 48]. The third-generation EGFR-TKI osimertinib specifically and irreversibly inhibits *EGFR* activating mutations and the *EGFR* T790M mutation[8].

The effectiveness of EGFR-TKIs is different depending on the subtype of *EGFR* mutation. In an integrated analysis of 12 clinical trials in advanced NSCLC patients with *EGFR* mutations, the exon 19 deletion mutation was significantly more sensitive to EGFR-TKIs than the L858R mutation regarding progeression free survival (PFS) (hazard ratio, HR, 0.69; 95% confidence interval, CI, 0.57 to 0.82; $P<0.001$), overall survival (OS) (HR, 0.61; 95% CI, 0.43 to 0.86; $P=0.005$), and overall response rate (ORR; odds ratio, OR, 2.14; 95% CI, 1.63 to 2.81; $P<0.001$)[49]. One reason why NSCLC with the exon 19 deletion has a higher sensitivity is the difference in the molecular structure of the two mutated forms of EGFR. EGFR with the exon 19 deletion mutation lacks 3–8 residues from the loop of the ATP-binding site, while L858R is located away from the ATP-binding site[50]. Another reason is that EGFR with the exon 19 deletion mutation has a structural change involving an essential residue of the tyrosine kinase domain, as the deletion mutation occurs at the α-helix. This change ensures that NSCLC with the exon 19 deletion mutation has a higher sensitivity to EGFR-TKIs than NSCLC with L858R[51]. Furthermore, the exon 19 deletion mutant activates downstream signaling even in monomeric form, but the L858R mutant does not activate downstream signaling unless it forms a dimer[52]. The autophosphorylation sites after dimer formation are also different between the two mutants, resulting in a difference in subsequent downstream signaling[53]. The uncommon *EGFR* mutations include the point mutation at codon 719 of exon 18 (G719X), E709X, exon 18 deletion mutation, exon 19 insertion mutation, exon 20 insertion mutation, S768I, and L861Q in exon 21. The frequency of the insertion mutation in exon 20 among the *EGFR* mutations is 5.8%, and the ORR is as low as 17% for first-generation EGFR-TKIs and 10% for afatinib[54-62]. On the other hand, the ORR for G719X is 32% for the first-generation EGFR-TKIs and 78% for afatinib[62]. The ORR for S768I and L861Q is 42% and 39%, respectively, for first-generation EGFR-TKIs and 100% and 56%, respectively, for afatinib[62]. An *EGFR* compound mutation is a double or multiple *EGFR* mutation, which was recently discovered by next-generation sequencing (NGS), and has been shown to lead to a poor prognosis[63].

An analysis of the metastatic pattern associated with NSCLC with an *EGFR* mutation showed brain metastasis was significantly more common in patients with an activated *EGFR* mutation (39.2%) than in patients with wild-type *EGFR* (28.2%) ($P=0.038$)[64]. It has been reported that brain metastasis is a poor prognostic factor in EGFR-TKI treatment in patients with an activated *EGFR* mutation[65]. The rate of progressive disease (PD) due to brain metastasis (central nervous system, CNS PD) during EGFR-TKI treatment is reported to be higher in patients with pretreatment brain metastasis than in patients without pretreatment brain metastasis[66-70]. EGFR-TKI migration into the cerebrospinal fluid (CSF) was first reported for erlotinib[71]. Compared to gefitinib, erlotinib migrates significantly more into the CSF ($P<0.0001$)[72]. Afatinib and osimertinib, which are next-generation EGFR-TKIs, are also reported to migrate into the CSF[73, 74].

Methods for detecting *EGFR* mutations include direct sequencing and a highly sensitive detection method involving polymerase chain reaction (PCR)[75-81]. The prevalence rate of *EGFR* mutations in non-adenocarcinoma, such as squamous cell carcinoma, is reported to be 0–5%[82-86]. Therefore, it is recommended to test for *EGFR* mutations using adenocarcinoma or specimens including a small amount of adenocarcinoma, especially regarding surgical specimens. However, this does not apply to small specimens such as bronchoscopic specimens and guided biopsy specimens. A surgical specimen, a bronchoscopic specimen, pleural effusion, or pericardial effusion can be used for analysis. Although the detection sensitivity of the direct sequencing method is assumed to be about 25%, the detection sensitivity of the peptide nucleic acid-locked nucleic acid (PNA-LNA) PCR Clamp method, Scorpion-amplification refractory mutation system (ARMS) method, Cycleave PCR method, PCR-invader method, Cobas method and the like is 1–5%[75, 78-81]. Additionally, it has been reported that the detection rates for cytology specimens are almost the

same among all the different methods[87, 88]. In addition to using tissue specimens, liquid biopsy to obtain cell-free DNA (cfDNA) is now being performed. A meta-analysis of *EGFR* mutation tests using serum cfDNA in patients with NSCLC (in which tissue specimens were used as the reference) revealed that the specificity of using cfDNA specimens was 0.96 and the sensitivity was 0.62[89]. Liquid biopsy is expected to be performed in order to carry out the T790M mutation test for EGFR-TKI-resistant patients. In addition, liquid biopsy is suitable for patients who cannot undergo invasive bronchoscopy or computed tomography (CT)-guided percutaneous lung biopsy or patients who need to be monitored for tolerance over time. An algorithm set out in the consensus statement of the International Association for the Study of Lung Cancer (IASLC) recommends that a review of the feasibility of re-biopsy precedes consideration of carrying out a liquid biopsy to obtain a plasma specimen for detecting a secondary T790M mutation[90]. Although a liquid biopsy is a biopsy with low invasiveness, false negative results are often a problem. Therefore, in the case of negative T790M results after liquid biopsy, the possibility of false negatives should be taken into consideration, and when tissue collection is possible due to disease progression, the presence of the T790M mutation should be re-assessed using tissue specimens. Recently, liquid biopsy using urine and saliva has also been reported[91, 92].

Common adverse events (AEs) of first- and second-generation EGFR-TKIs are mainly skin disorders, paronychia, diarrhea, and, less frequently but importantly, interstitial lung disease (ILD) (Common Terminology Criteria for Adverse Events, CTCAE grade 3 or more, 0.6–2.2%)[93]. The incidence of ILD is greatly affected by racial differences, being 2.5% for Asians and 0.9% for non-Asians. Although the mechanism by which EGFR-TKIs induce ILD has not been sufficiently clarified, a mechanism that prevents recovery of epithelial injuries and directly causes lung injuries has been reported[94]. The risk factors for ILD are reported to be the presence of interstitial pneumonia, having a history of smoking, being male, poor performance status (>2), and previous radiation therapy[95, 96]. Studies on ILD in Asia (including Japan) revealed that the mean mortality rate was 44.3%, and 75% for ILD with diffuse alveolar damage (DAD)[97]. High doses of methylprednisolone and immunosuppressive drugs are used for DAD-type ILD while oral corticosteroid treatment is used for non-DAD-type ILD[98]. Skin disorders induced by EGFR-TKIs include acneiform rash, xerosis, erythema, photosensitivity, fissures and cracks, hyperpigmentation, telangiectasia, and pruritus[99]. Regarding the mechanism of EGFR-TKI skin disorder development, EGFR-TKIs may increase the expression of $p27^{KIP1}$[100-102], a cyclin-dependent kinase inhibitor, which leads to cell cycle arrest of keratinocytes at the G1 phase, and they may induce the expression of members of the C-C motif chemokine ligand (CCL) and C-X-C motif chemokine (CXCL) families, which exacerbate skin inflammation[103, 104]. Anti-inflammatory antibiotics and corticosteroids are administered locally for grade 1 skin disorders and orally for grade 2 skin disorders[105]. EGFR is expressed even in the gastrointestinal tract[106]. EGFR-TKI-related diarrhea is thought to be caused by overproduction of chloride by EGFR-TKI[107], and diarrhea is also thought to be induced by factors such as change of intestinal motility, colonic crypt damage, and change in the intestinal microflora[108]. Loperamide can be used to treat diarrhea. The frequency of AEs such as skin disorders or diarrhea is highest for gefitinib, followed by erlotinib and afatinib, while liver dysfunction is more common after gefitinib treatment[93]. In the case of grade 1/2 diarrhea caused by afatinib, 4 mg oral loperamide is administered immediately, and it is increased by 2 mg each time the patient has diarrhea. The maximum dose of loperamide is 20 mg/day[109]. On the other hand, AEs after third-generation EGFR-TKI are less frequent because its effect on wild-type EGFR was developed to be limited, although it acts on activated *EGFR* mutations and the T790M mutation[110].

2.1.1. Gefitinib (IRESSA®)

Gefitinib is synonymous with N-(3-Chloro-4-fluorophenyl)-7-methoxy-6-(3-(4-morpholinyl)propoxy)-4-quinazolinamine. In the era when patients were not limited to those with an activated *EGFR* mutation, a phase III trial involving previously treated advanced NSCLC patients (ISEL trial) did not show that gefitinib significantly improved survival compared to placebo[111]. Subgroup analysis showed that the therapeutic effect of gefitinib was high in non-smokers and Asians. Indeed, a randomized phase II trial comparing 250 and 500 mg gefitinib once daily in patients with previously treated NSCLC (IDEAL 1 trial) showed meaningful antitumor activity of gefitinib[112]. Multivariate analysis showed that being female and having adenocarcinoma were independent prognostic factors associated with an objective improvement. Although a phase III trial comparing gefitinib and docetaxel for previously treated NSCLC patients in Japan (V-15-32 trial) did not confirm the non-inferiority of gefitinib compared to docetaxel[113], and a phase III trial comparing gefitinib with gemcitabine and cisplatin in previously untreated never-smokers with lung adenocarcinoma (First-SIGNAL trial) did not show the superior OS[114], another phase III trial comparing gefitinib and docetaxel for previously treated NSCLC patients (INTEREST trial) confirmed the non-inferiority of gefitinib compared to docetaxel[115]. As described above, it was suggested that the selection of patients is important for gefitinib treatment. Therefore, the next phase III trial was performed in chemo-naïve NSCLC patients who were non- or light smokers and had adenocarcinoma, which were reported to be predictive markers of response to gefitinib (IPASS trial)[31]. This trial firstly revealed a significant improvement of PFS in the gefitinib group compared to the carboplatin plus paclitaxel group (HR for progression or death, 0.74; 95% CI, 0.65 to 0.85; $P<0.001$)[31]. A crossover was observed in the Kaplan-Meier survival curves, and it turned out that the presence of an activated *EGFR* mutation was thought to be the cause. Here, as a result of vigorous research, it is clear that the effect of EGFR-TKIs is due to the presence of an activated *EGFR* mutation[5]. Subsequently, two phase III clinical trials in Japan were the first to examine the effect of gefitinib on NSCLC with an activated *EGFR* mutation, rather than NSCLC involving specific clinical background factors (e.g., adenocarcinoma or nonsmokers). These phase III trials

comparing gefitinib with carboplatin plus paclitaxel (NEJ002 trial) or cisplatin plus docetaxel (WJTOG3405 trial) in chemo-naïve NSCLC patients with an activated *EGFR* mutation revealed that gefitinib showed superiority regarding PFS (HR, 0.30; 95% CI, 0.22 to 0.41; $P<0.001$; 10.8 vs. 5.4 months and HR, 0.489; 95% CI, 0.336 to 0.710; $P<0.0001$; 9.2 vs. 6.3 months, respectively)[116, 117]. Regarding OS, there was no significant difference between the two groups, but this was caused by crossover after the second treatment.

In recent years, clinical trials have examined combination therapy involving gefitinib and other drugs. Although a therapeutic strategy involving the use of chemotherapy combined with an EGFR-TKI after progression (beyond PD) is thought to be theoretically effective[118], a phase III trial confirming the significant effect of adding cisplatin and pemetrexed to gefitinib after exacerbation (IMPRESS trial) showed that the PFS did not differ between the gefitinib plus chemotherapy group and the placebo plus chemotherapy group and the OS was significantly lower in the gefitinib plus chemotherapy group[119]. However, subsequent analysis confirmed that OS was significantly improved in the gefitinib plus chemotherapy group when the patients were restricted to the T790M-positive patients at randomization[120]. On the other hand, a randomized phase II trial of concurrent versus alternating gefitinib and carboplatin/pemetrexed in previously untreated NSCLC patients with an activated *EGFR* mutation (NEJ005/TCOG0902 trial) revealed that the concurrent regimen showed superiority regarding OS (HR, 0.58; 95% CI, 0.34 to 0.97; $P=0.036$; 41.9 vs. 30.7 months)[121]. A phase III trial comparing gefitinib plus carboplatin and pemetrexed and gefitinib monotherapy was conducted in untreated stage III/IV or postoperative recurrent non-squamous NSCLC patients with an active *EGFR* mutation (NEJ009 trial)[122]. In the gefitinib monotherapy group, platinum-based chemotherapy was used after PD. The order of analysis of primary endpoints was PFS 1, PFS 2, and OS using the gatekeeping method. PFS 1 was defined as PFS up to the first PD (PD 1), and in the gefitinib monotherapy group PFS 2 was defined as PFS up to PD after the initiation of second-line chemotherapy (PD 2). On the other hand, in the gefitinib plus chemotherapy group, PFS2 was defined as PFS up to PD 1. The median PFS 1 was significantly better in the gefitinib plus chemotherapy group than in the gefitinib monotherapy group (HR, 0.494; 95% CI, 0.391 to 0.625; $P<0.001$; 20.9 vs. 11.2 months). On the other hand, the median PFS 2 did not show a significant difference between the gefitinib plus chemotherapy group and gefitinib monotherapy group (HR, 0.966; 95% CI, 0.766 to 1.220; $P=0.774$; 20.9 vs. 20.7 months). The median OS was significantly better in the gefitinib plus chemotherapy group than in the gefitinib monotherapy group (HR, 0.695; 95% CI, 0.520 to 0.927; $P=0.013$; 52.5 vs. 38.8 months). AEs in the gefitinib plus chemotherapy group were relatively severe, with 65.1% being grade 3–5, while only 31.4% in the gefitinib monotherapy group were grade 3–5. In particular, blood toxicity was remarkable in the gefitinib plus chemotherapy group. However, treatment discontinuation due to AEs was 9.9% in the gefitinib plus chemotherapy group and 10.7% in the gefitinib monotherapy group, and there was no significant difference in patient condition at PD 1 between the two groups. Attention must be paid to the fact that performance status at PD 2 was declining in the gefitinib monotherapy group. As performance status becomes worse, subsequent use of chemotherapy is impossible, so using gefitinib plus chemotherapy as early-line treatment may be important from the viewpoint of ensuring that cytotoxic anticancer drugs can be used.

2.1.2. Erlotinib (TARCEVA®)

Erlotinib is synonymous with N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine. A phase II trial was conducted on 57 previously treated NSCLC patients that were positive for EGFR mutation by immunostaining[123]. The trial revealed that the ORR was 12.3% (95% CI, 5.1% to 23.7%) and the MST was 8.4 months (95% CI, 4.8 to 13.9 months)[123]. Intriguingly, this trial showed that the possibility of survival correlated with the appearance of skin disorders and their severity. A phase III trial comparing erlotinib and best supportive care (BSC) in previously treated NSCLC patients (BR.21 trial) in which patients were not selected for *EGFR* mutation status showed that both OS and PFS were significantly better in the erlotinib group than in the BSC group[6]. This result, which is different from the result of the ISEL trial, may have been due to the fact that 150 mg of erlotinib (the maximum tolerated dose (MTD)) was used in the BR.21 trial, whereas 250 mg of gefitinib (one-third of the MTD) was used in the ISEL trial and that affinity for EGFR is different between the two drugs[124]. Interim analysis of a post-marketing phase IV trial (TRUST trial), in which 7000 patients in 52 countries were registered, reported the same good tolerability as in the BR.21 trial. Two phase III trials confirming the effect of erlotinib compared with carboplatin plus gemcitabine (OPTIMAL/CTONG-0802 trial) and cisplatin or carboplatin plus docetaxel or gemcitabine (EURTAC trial) in chemo-naïve NSCLC patients with an activated *EGFR* mutation revealed that erlotinib showed superiority regarding PFS and ORR[125, 126]. To compare first-generation EGFR-TKIs with each other, a phase III trial comparing gefitinib and erlotinib in patients with previously treated advanced lung adenocarcinoma (WJOG5108L trial) was conducted and did not demonstrate noninferiority of gefitinib to erlotinib[127]. There are very promising results regarding combination treatment involving chemotherapy. A randomized phase II trial comparing erlotinib plus bevacizumab with erlotinib alone in patients with advanced non-squamous NSCLC harboring an *EGFR* mutation (JO25567 trial) revealed that erlotinib plus bevacizumab showed superiority regarding PFS (HR, 0.54; 95% CI, 0.36 to 0.79; $P=0.0015$; 16.0 vs. 9.7 months)[128]. In this trial, bevacizumab-related AEs such as grade 3 hypertension (60%) and grade 1/2 hemorrhagic events (69%) were observed in the erlotinib plus bevacizumab group. A subsequent phase III trial comparing erlotinib plus bevacizumab with erlotinib alone in patients with advanced non-squamous NSCLC harboring an *EGFR* mutation (NEJ026 trial) revealed that erlotinib plus bevacizumab showed superiority regarding PFS (HR, 0.605; 95% CI, 0.417 to 0.877; $P=0.01573$; 16.9 vs. 13.3 months)[129]. Although the OS data from the JO25567 trial (which were reported at the ASCO 2018 annual meeting) indicated that erlotinib plus bevacizumab showed no significant improvement regarding OS (HR, 0.81; 95% CI, 0.53 to 1.23; $P=0.3267$; 47.0 vs. 47.4 months)[130], erlotinib plus bevacizumab may be a treatment option, especially in cases of pleural effusion.

2.1.3. Afatinib (GIOTRIF®)

Afatinib is synonymous with (S,E)-N-(4-(3-Chloro-4-fluorophenylamino)-7-(tetrahydrofuran-3-yloxy)quinazolin-6-yl)-4-(dimethylamino)but-2-enamide. Afatinib is an irreversible inhibitor of members of the HER family (including EGFR). A phase II trial confirming the effect of afatinib (LUX-Lung 2 trial) revealed that ORR was observed in 66% of patients with a common *EGFR* mutation[131]. Two phase III trials confirming the effect of afatinib compared with cisplatin plus pemetrexed (LUX-Lung 3 trial) and cisplatin plus gemcitabine (LUX-Lung 6 trial) in chemo-naïve NSCLC patients with an activated *EGFR* mutation revealed that afatinib showed superiority regarding PFS[132, 133] (Table 2). Furthermore, integrated analysis of the LUX-Lung 3 and 6 trials showed that afatinib significantly prolonged the OS compared to chemotherapy (HR, 0.81; 95% CI, 0.66 to 0.99; *P*=0.037; 27.3 vs. 24.3 months)[134]. An integrated analysis of the LUX-Lung 2, 3 and 6 trials showed that the ORR in NSCLC patients harboring an uncommon *EGFR* mutation (G719X at exon 18, S768I at exon 20, and L861Q at exon21) treated with afatinib was 78%, 100%, and 56%, respectively[62], while the ORR in NSCLC patients with a G719X, S768I, and L861Q mutation treated with a first-generation EGFR-TKI was only 32%, 42%, and 39%, respectively[135]. In addition, afatinib is reported to be effective even against leptomeningeal carcinomatosis, particularly in patients with an uncommon mutation[73]. A randomized phase IIb trial comparing afatinib and gefitinib in patients with NSCLC harboring an *EGFR* mutation (LUX-Lung 7 trial) revealed that afatinib showed superiority regarding PFS (HR, 0.73; 95% CI, 0.57 to 0.95; *P*=0.0073; 11.0 vs. 10.9 months)[7]. AEs induced by afatinib can be managed with rest or the reduction of the afatinib dosage (based on pre-defined criteria) and with appropriate active supportive care. Considering that the pharmacokinetics of afatinib are highly variable among individuals, it is necessary to carefully observe the frequency and severity of AEs at the beginning of administration and reduce afatinib to the optimum dose. In particular, it is important to initiate supportive care early for diarrhea, rash/acne, stomatitis, and nail abnormalities.

2.1.4. Osimertinib (TAGRISSO®)

Osimertinib is synonymous with N-(2-((2-(Dimethylamino)ethyl)(methyl)amino)-4-methoxy-5-((4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl)amino)phenyl)prop-2-enamide monomethanesulfonate. Osimertinib was developed as an irreversible EGFR-TKI for NSCLC harboring wild-type and mutant *EGFR*, and the preclinical data showed that osimertinib had a strong inhibitory effect against activated *EGFR* mutations and T790M compared to its effect against wild-type EGFR[8]. A dose escalation test and a dose expansion test associated with the phase I/II clinical trials of osimertinib (AURA 1/AURA 2 trials) in *EGFR* mutation-positive NSCLC patients that became resistant to EGFR-TKI were carried out[110]. The ORR of T790M mutation-positive cases was 61% (95% CI, 52 to 70), the median PFS was 9.6 months (95% CI, 8.3 to not reached), the ORR of the negative cases was 21% (95% CI, 12 to 34), and the median PFS was 2.8 months (95% CI, 2.1 to 4.3). Subsequently, a phase III trial confirming the effect of osimertinib compared with cisplatin plus pemetrexed in NSCLC patients with the *EGFR* T790M mutation who were resistant to first-line EGFR-TKI (AURA3 trial) revealed that osimertinib showed superiority regarding PFS (HR, 0.30; 95% CI, 0.23 to 0.41; *P*<0.001; 10.1 vs. 4.4 months)[35]. Intriguingly, osimertinib is reported to transfer to the CNS at high concentrations in an experimental model[74, 136]. Indeed, the AURA trial showed that osimertinib showed superiority regarding PFS in patients who had CNS metastasis at the beginning of the study (HR, 0.32; 95% CI, 0.21 to 0.49; 8.5 vs. 4.2 months)[35]. Another phase III trial confirming the effect of osimertinib compared with first-generation EGFR-TKIs in untreated NSCLC patients with an activated *EGFR* mutation (FLAURA trial) revealed that osimertinib showed superiority regarding PFS (HR, 0.46; 95% CI, 0.37 to 0.57; *P*<0.001; 18.9 vs. 10.2 months)[9]. The common any grade and grade 3/4 AEs are summarized in Table 3. In countries where osimertinib can be used as a first-line EGFR-TKI, it is desirable to use osimertinib in first-line treatment. Future clinical trials will determine which treatments out of erlotinib plus bevacizumab, afatinib, dacomitinib, and osimertinib are suitable for the first-line treatment of NSCLC harboring an *EGFR* mutation[137].

2.1.5. Others

Dacomitinib is synonymous with (2E)-N-(4-((3-chloro-4-fluorophenyl)amino)-7-methoxy-6-quinazolinyl)-4-(1-piperidinyl)-2-butenamide. A phase III trial comparing dacomitinib, a novel second-generation EGFR-TKI, and gefitinib in patients with advanced NSCLC harboring an activated *EGFR* mutation (ARCHER trial) showed that the OS was significantly better in the dacomitinib group than in the gefitinib group (HR, 0.76; 95% CI, 0.582 to 0.993; *P*=0.0438; 34.1 vs. 26.8 months)[138].

Rociletinib is synonymous with N-(3-((2-(4-(4-acetylpiperazin-1-yl)-2-methoxyanilino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)phenyl)prop-2-enamide and oral mutant-selective third-generation EGFR-TKI. A phase I/II trial of rociletinib in patients with *EGFR*-mutant NSCLC with acquired resistance to first- or second-generation EGFR-TKI (TIGER-X trial) showed that the ORR of T790M mutation-positive cases was 59% (95% CI, 45 to 73)[139]. The most common grade 3AE was hyperglycemia. Unfortunately, even for third-generation EGFR-TKIs, resistant mutations appear within about 1 year, and the C797S mutation was reported to be one of the resistant mutations[140, 141]. Brigatinib (See also 2.2.4.), which acts as an EGFR-TKI and an ALK-TKI, was reported to be effective against the resistance C797S mutation *in vitro* and *in vivo*[142]. Other resistance mechanisms besides T790M have also been reported, including mesenchymal–epithelial transition factor *(MET)* amplification, *HER2* amplification, hepatocyte growth factor (*HGF*) overexpression, CRK-like proto-oncogene (*CRKL*) gene amplification, *PI3K* mutation, *BRAF* V600E mutation, *MAPK1* amplification, loss of phosphatase and tensin homolog (PTEN) expression, transformation to small cell lung cancer (SCLC), and epithelial–mesenchymal transition (EMT) induced by AXL activation, decreased MED12 expression, and TGF-β-IL68[143-154]. Further research and clinical trials regarding overcoming tolerance are needed.

2.2. ALK-TKI

An *EML4-ALK* fusion gene was detected in adenocarcinoma cells in 2007[32]. This *EML4-ALK* fusion

gene encoded EML4 (a microtubule-associated protein) and the intracellular tyrosine kinase domain of the ALK receptor tyrosine kinase, resulting in constitutive activation of the ALK receptor and oncogenesis. *ALK* fusion genes are often detected in young or nonsmoking patients[155]. However, as an important point, *ALK* fusion genes are also often detected in lung cancer specimens from smokers and elderly people. Therefore, it is impossible to predict the presence or lack of *ALK* fusion genes using only these clinical background details[156]. The *EML4-ALK* fusion gene is the most frequently observed *ALK* fusion gene, but kinesin family 5B (*KIF5B*)-*ALK* and TRK-fused gene (*TFG*)-*ALK* are also observed[157, 158]. Inamura et al. reported that 6 out of 11 cases of *EML4-ALK*-positive lung cancer were the acinar-predominant type and the other 5 cases were the papillary-predominant type[159]. All 11 cases were positive for thyroid transcription factor-1 (TTF-1). On the other hand, Rodig et al. reported that the solid-predominant pattern is more frequent among bronchiolar-alveolar carcinomas (BACs), acinar, papillary, and solid adenocartinoma[160]. At the cellular level, 82% of lung cancer with an *ALK* fusion gene was occupied with signet ring cells[160].

ALK-TKIs dramatically improve prognosis of NSCLC with an *ALK* fusion gene. ALK-TKIs include the first-generation crizotinib, and the second-generation alectinib and ceritinib. However, in most cases, patients develop tolerance to crizotinib within 1 year of treatment[15]. Resistance to ALK-TKIs is classified as initial tolerance or acquisition tolerance. Acquisition tolerance is further divided into ALK dominant tolerance and ALK non-dominant tolerance[37]. ALK dominant tolerance includes secondary mutation of the *ALK* gene and amplification of the *ALK* gene. On the other hand, ALK non-dominant tolerance includes activation of bypass signaling by activation of *EGFR*, Kirsten rat sarcoma (*KRAS*), insulin-like growth factor 1 receptor (*IGF-1R*) signaling, *KIT*, and *MET* amplification[161-163]. The most common type of tolerance involves a secondary mutation such as C1156Y and L1196M[37]. *In vitro* experiments showed that second-generation ALK-TKIs such as alectinib, ceritinib, and brigatinib have sensitivity to *ALK* fusion gene-positive NSCLC cell lines harboring a secondary mutation such as L1196M or G1269A[164-166]. However, even after alectinib treatment, secondary mutations such as V1180L, I1171T, and G1202R were observed[167, 168]. Ceritinib also induces secondary mutations such as F1174C, F1174V, and G1202R[165]. Third-generation ALK-TKIs such as lorlatinib have been designed to be effective against some of these mechanisms of tolerance. Furthermore, pemetrexed-containing regimens may be considered in the treatment sequence, because reports showed that pemetrexed was effective against *ALK* mutation-positive lung cancer[169, 170].

Methods for detecting *ALK* fusion genes include the fluorescence in situ hybridization (FISH) method, immunohistochemistry (IHC) method, and reverse transcription (RT)-PCR method. The prevalence rate of *ALK* fusion genes in non-adenocarcinoma, particularly in squamous cell carcinoma, is reported to be quite low[159, 171-176]. Therefore, it is recommended to test for *ALK* fusion genes using an adenocarcinoma specimen or a specimen including a small amount of adenocarcinoma. A surgical specimen, a specimen obtained by bronchoscopy, pleural effusion, or pericardial effusion can be used for analysis. In the case of liquid specimens, it is recommended to create cell blocks to perform the FISH or IHC method. The FISH method is the most established method for diagnosis of *ALK* fusion gene-positive cancer[177, 178]. But the FISH method is expensive and it is inappropriate as a screening test because of insufficient sensitivity and specificity[179]. On the other hand, the IHC method is suitable for screening. A highly sensitive IHC method has been developed because the expression of the *ALK* gene is very low and detection by conventional staining methods is difficult[158, 159, 171, 180, 181]. Therefore, it is recommended that screening is performed using the high-sensitivity IHC method and confirmed using the FISH method.

AEs of ALK-TKIs include diarrhea, vomiting, liver dysfunction, and vision disorder. The frequency of AEs is highest for ceritinib, followed by crizotinib and alectinib[182]. Gastrointestinal symptoms (at any CTCAE grade) induced by crizotinib were observed in about 50% of cases[10]. On the other hand, ceritinib induced more gastrointestinal symptoms than crizotinib and alectinib[183].

2.2.1. Crizotinib (XALKOLI®)

Crizotinib was initially developed as an c*MET* inhibitor and is a multi-molecular targeted drug that can inhibit *ALK* as well as multiple phosphorylating enzymes such as *MET* and *ROS1*[184]. A phase III trial confirming the effect of crizotinib compared with pemetrexed or docetaxel in NSCLC patients with an *ALK* fusion gene who relapsed after a first-line platinum-based regimen (PROFILE1007 trial) revealed that crizotinib showed superiority regarding PFS (HR, 0.49; 95% CI, 0.37 to 0.64; $P<0.001$; 7.7 vs. 3.0 months) and ORR (65% vs. 20%; $P<0.001$)[10]. Another phase III trial confirming the effect of crizotinib compared with cisplatin plus pemetrexed on untreated NSCLC patients with an *ALK* fusion gene (PROFILE1014 trial) revealed that crizotinib showed superiority regarding PFS (HR, 0.45; 95% CI, 0.35 to 0.60; $P<0.0001$; 10.9 vs. 7.0 months) and ORR (74% vs. 45%; $P<0.001$)[177]. Visual impairment and gastrointestinal symptoms were frequent AEs. Many patients had light and dark adaptation disorders with symptoms such as the persistence of afterimages. However, most visual impairment was transient and mild. Nausea or vomiting was often seen within 7 days of the first administration, particularly within 2 days. Most gastrointestinal toxicities can be treated according to the symptoms. Careful monitoring is needed for ILD, liver dysfunction, and QT interval prolongation as these AEs can be severe and fatal.

Oligo-progressive disease involves TKI failure in limited metastatic lesions (such as bone or brain metastases) with continued response in other lesions[185]. For oligo-progressive disease, it has been reported that the PFS can be extended by continuing TKI treatment (beyond PD) as much as possible while adding in local treatment[185, 186]. Indeed, integrated analysis of a phase I trial (PROFILE1001 trial) and a phase II trial (PROFILE1005 trial) showed that the MST in the beyond PD group was longer than that in the non-beyond PD group after the occurrence of crizotinib failure (HR, 0.27; 95% CI, 0.17 to 0.42; $P<0.0001$; 16.4 vs. 3.9 months)[187]. However, effective second-generation ALK-TKIs such as alectinib or ceritinib can be used instead of continuous crizotinib.

It has been reported that crizotinib may have an effect if metastasis is involved in alectinib-induced resistance[188].

2.2.2. Alectinib (ALECENSA®)

Alectinib is a second-generation ALK-TKI and its selectivity to ALK is remarkably high compared to that of crizotinib. Alectinib is effective even after crizotinib-resistant *ALK* mutations such as L1196M and C1156Y have developed[36]. A phase II trial of alectinib in NSCLC patients with an *ALK* fusion gene previously treated with crizotinib demonstrated that the ORR of alectinib was 50% (95% CI, 41 to 59) and the PFS was 8.9 months (95% CI, 5.6 to 11.3)[189]. Intriguingly, this trial also showed that the control rate of CNS metastasis was 83% (95% CI, 74 to 91)[189]. A phase III trial confirming the effect of alectinib compared with crizotinib in untreated NSCLC patients with an *ALK* fusion gene (J-ALEX trial) revealed that alectinib showed superiority regarding PFS (HR, 0.34; 99.7% CI, 0.17 to 0.71; $P<0.0001$; not reached vs. 10.2 months) at the second interim analysis[38]. A recent global phase III trial confirming the effect of alectinib compared with crizotinib in untreated NSCLC patients with an *ALK* fusion gene (ALEX trial) also revealed that alectinib showed superiority regarding PFS (HR, 0.47; 95% CI, 0.34 to 0.65; $P<0.001$; not reached vs. 11.1 months) and CNS progression (HR, 0.16; 95% CI, 0.10 to 0.28; $P<0.001$; 12% vs. 45%)[14]. As alectinib has highly selective inhibitory activity against ALK, most of its AEs are grade 1. In summary, alectinib is considered the first choice for first-line treatment of *ALK* fusion gene-positive NSCLC.

2.2.3. Ceritinib (ZYKADIA®)

Ceritinib is a second-generation ALK-TKI and its selectivity to ALK is remarkably high compared to that of crizotinib. Ceritinib is not effective for cases involving crizotinib-resistant *ALK* mutations such as G1202R and F1174C but it is effective for cases involving L1196M, G1296A, I1171T, and S1206Y mutations[165]. A phase III trial confirming the effect of ceritinib compared with cisplatin or carboplatin plus pemetrexed in untreated NSCLC patients with an *ALK* fusion gene (ASCEND-4 trial) revealed that ceritinib showed superiority regarding PFS (HR, 0.55; 95% CI, 0.42 to 0.73; $P<0.00001$; 16.6 vs. 8.1 months)[183]. Another phase III trial confirming the effect of ceritinib compared with pemetrexed, docetaxel, or no treatment in NSCLC patients with an *ALK* fusion gene who had received chemotherapy including platinum doublet regimens (ASCEND-5 trial) revealed that ceritinib showed superiority regarding PFS (HR, 0.49; 95% CI, 0.36 to 0.67; $P<0.0001$; 5.4 vs. 1.6 months)[190] (Table 4). Ceritinib is relatively toxic and the frequent AEs include diarrhea, nausea, vomiting, dehydration symptoms, liver dysfunction, abdominal pain, and hypophosphatemia.

Ceritinib is reported to be sensitive to the secondary mutation I1171N/S/T induced by crizotinib and alectinib[167, 191-193]. Indeed, a phase II trial evaluating efficacy of ceritinib in patients with alectinib-refractory *ALK* rearrangement-positive NSCLC (ASCEND-9 trial) revealed that the ORR was 25% (95% CI, 8.7 to 49.1) and the PFS was 3.7 months (95% CI, 1.9 to 5.3)[194]. Ceritinib may be one of the treatment options after use of crizotinib or alectinib.

2.2.4. Others

Brigatinib, which is a second-generation ALK-TKI, shows activity against mutated ROS1 and EGFR including EGFR with the T790M mutation[195]. A phase I/II trial of brigatinib in NSCLC patients with an *ALK* fusion gene previously treated with crizotinib demonstrated that the ORR of brigatinib was 62% (95% CI, 50 to 73)[195]. The most common grade 3–4 AEs were increased lipase concentration (9%), dyspnea (6%), and hypertension (5%)[195]. Another phase II trial evaluating the efficacy of brigatinib in patients with crizotinib-refractory *ALK* fusion gene-positive NSCLC revealed that brigatinib had sufficient antitumor activity and prolonged the PFS[196]. Lorlatinib, which is a third-generation ALK-TKI, shows activity against ALK with the secondary mutation G1202R[197]. A multicenter phase I trial of lorlatinib in NSCLC patients with *ALK* or *ROS1* rearrangement showed that the ORR in NSCLC patients with an *ALK* mutation was 46% (95% CI, 31 to 63)[198]. Dose-limiting toxicity (DLT) involved grade 2 neurocognitive AEs such as slowed speech and mentation and word-finding difficulty[198]. Entrectinib, which is a third-generation ALK-TKI, shows activity against ROS1 and TrkA/B/C. The combined results of two phase I trials of entrectinib in patients harboring neurotropic tropomyosin receptor kinase *(NTRK)1/2/3*, *ROS1*, or *ALK* fusion genes demonstrated that the ORR for entrectinib was 42%[199]. Entrectinib did not cause AEs of grade 3 or more [199]. Unfortunately, if at least two mutations occur within the ALK kinase region, the tumors are also resistant to brigatinib and lorlatinib. The E1210K+S1206C and E1210K+D1203N mutations were found in patients who became resistant to brigatinib[37]. Intriguingly, it has also been revealed that these double mutations leads to high susceptibility to crizotinib[16].

Ensartinib is a third-generation ALK-TKI. A phase I/II clinical trial of ensartinib in patients with *ALK*-positive NSCLC showed that the ORR was 80% (95% CI, 54.8 to 93) and PFS was 26.2 months (95% CI, 7.6 to not estimable) in ALK-TKI-naïve patients[200].

On the other hand, heat shock protein (HSP)-90 inhibitors are novel therapeutic agents for NSCLC harboring an *ALK* fusion gene. It has been reported that IPI-504[201], STA-9090 (ganetespib)[202], and AUY922[203] were effective against NSCLC with an *ALK* fusion gene. HSP90 is a protein molecular chaperone and is highly expressed in cancer cells and tumor tissues. It stabilizes many cancer-related factors and maintains the survival and proliferation of cancer cells. HSP90 inhibitors structurally destabilize proteins and induce cancer growth arrest and apoptosis.

2.3. ROS1-TKI

The *ROS1* fusion gene is a rare genetic abnormality found in 1% of NSCLC patients[204]. The *ROS1* fusion gene is developed by the fusion of the *ROS1* gene with various partner genes. The ROS1 fusion protein encoded by the *ROS1* fusion gene causes constitutive activation of downstream signaling involved in cell proliferation and survival[204]. The clinical features associated with NSCLC patients harboring a *ROS1* fusion gene are being young, being a non-smoker, and having adenocarcinoma[11].

Methods for detecting *ROS1* fusion genes include RT-PCR, IHC, FISH, and NGS methods. In the future, multiple diagnoses using NGS will be clinically applied and it seems that many genetic abnormalities will be able to be diagnosed

at the same time. Until then, we should collect abundant and good quality specimens and test for *ROS1* fusion genes to ensure early diagnosis and early treatment. Currently, IHC leads to many false negatives and RT-PCR is thought to be the most appropriate test.

For *ROS1* fusion gene-positive lung cancer, high efficacy of crizotinib has been reported[33, 205], but similar to *ALK* fusion gene-positive lung cancer, *ROS1* mutations (G2032R, D2033N, S1986Y, and S1986F), which are considered as the cause of resistance to crizotinib, have been found[206-209].

2.3.1. Crizotinib (XALKOLI®)

See also 2.2.1. A phase I trial of crizotinib in NSCLC patients with *ROS1* rearrangement showed that the ORR was 72% (95% CI, 58 to 84) and the PFS was 19.2 months (95% CI, 14.4 to not reached)[33]. The AEs of crizotinib were almost similar to those seen in NSCLC patients harboring an *ALK* fusion gene. A subsequent phase II trial of NSCLC patients harboring a *ROS1* fusion gene was conducted in Asia (OO12-01 trial) and revealed that the ORR of crizotinib was 69.3% (95% CI, 60.5 to 77.2%)[205]. (Table 5).

2.3.2. Others

A multicenter phase I trial of lorlatinib in NSCLC patients with *ALK* or *ROS1* rearrangement showed that the ORR in NSCLC patients with a *ROS1* rearrangement was 50% (95% CI, 21 to 79)[198].

2.4. BRAF-TKI

The *BRAF* mutation is found in 1–2% of NSCLC patients. 56.8% of *BRAF* mutations are V600E and 43.2% are non-V600E[210]. The clinical features associated with NSCLC patients with *BRAF* V600E are being female, having adenocarcinoma with a micropapillary component, and having poor prognosis[210, 211].

2.4.1. Dabrafenib (TAFINLAR®)

Dabrafenib is a BRAF kinase inhibitor that specifically acts on cancers with *BRAF* mutations. A phase I trial showed efficacy in NSCLC patients with a *BRAF* mutatation[212]. A subsequent phase II trial of dabrafenib in advanced NSCLC patients with the *BRAF* V600E mutation showed that the ORR was 33% (95% CI, 23 to 45)[213]. The most common AEs of grade 3 or above were cutaneous squamous-cell carcinoma (12%), asthenia (5%), and basal-cell carcinoma (5%).

2.4.2. Trametinib (MEKINIST®)

Trametinib is an oral mitogen-activated protein kinase kinase (MEK) inhibitor. In *BRAF*-mutant metastatic melanoma, combination therapy involving dabrafenib and trametinib improved the ORR, PFS, and OS compared to BRAF inhibitor monotherapy[214-216]. A phase II trial of combination therapy involving dabrafenib and trametinib in 57 previously treated NSCLC patients with the *BRAF* V600E mutation was performed[13]. The ORR, which was the primary endpoint, was 66.7% and the median PFS was 9.7 months.

2.5. RET-TKI

The *RET* rearrangement is found in 1–2% of NSCLC patients[204]. *RET* encodes a receptor tyrosine kinase and is mostly rearranged into the fusion gene *KIF5B-RET*[217-220]. The clinical features associated with NSCLC patients with *RET* rearrangement are being young, being a non-smoker, and having adenocarcinoma[221, 222].

2.5.1. Vandetanib (CAPRELSA®)

Vandetanib is an orally available multiple receptor TKI of VEGF, EGFR, and RET. A phase I trial showed efficacy in patients with NSCLC[223]. A subsequent phase II trial of vandetanib in previously treated NSCLC patients with *RET* rearrangement (LURET trial) showed that the ORR was 53% (95% CI, 28 to 77)[224]. The PFS was 4.7 months (95% CI 2.8 to 8.5). The most common grade 3/4 AEs were hypertension (58%), diarrhea (11%), rash (16%), dry skin (5%), and QT prolongation (11%). Another phase II trial of vandetanib in previously treated NSCLC patients with *RET* rearrangement showed that the ORR was 18%[225]. The PFS and OS were 4.5 and 11.6 months, respectively. The most common grade 3 AEs were hypertension (17%) and QT prolongation (11%).

2.5.2. Others

LOXO-292 is a novel highly selective RET inhibitor and is effective for activating *RET* fusions/mutations as well as potential resistance mutations[226]. A phase I trial of LOXO-292 in *RET* fusion gene-positive and *RET* mutation-positive NSCLC patients (LIBRETTO-001 trial) showed that the ORR was 69% (95% CI, 50 to 84)[227].

3. MOLECULAR THERAPY TARGETED AGAINST ANGIOGENESIS

It is well known that tumor growth is dependent on neovessels[228]. New blood vessels supply oxygen, nutrition, growth factors, etc. to the cancer, work to maintain homeostasis, and help the development of cancer cells[229, 230]. Additionally, it has been reported that the expression of tumor angiogenic factors correlate with malignancy[17-19]. When tumor cells are exposed to hypoxic conditions, VEGF, especially VEGF-A, which plays a crucial role in angiogenesis, is secreted by tumor cells[231, 232]. VEGF-A binds to its receptors in vascular endothelial cells, primarily VEGF receptor (VEGFR)-2, and promotes the proliferation, migration, and survival of vascular endothelial cells. Bevacizumab is a human monoclonal antibody against VEGF-A, whereas ramucirumab is a human recombinant monoclonal immunoglobulin (Ig) G1 antibody against VEGFR-2. In the preclinical setting, murine anti-VEGF monoclonal antibody suppressed angiogenesis and growth of a human tumor xenograft[233, 234]. Similarly, in preclinical studies, anti-VEGFR-2 antibody inhibited VEGF-induced signaling, angiogenesis, and tumor growth[235-239].

VEGF is also involved in the mechanism of malignant pleural effusion, which is known to be a predictor of poor prognosis. VEGF and VEGFR inhibitors have been shown to inhibit the production of pleural effusion[240-243]. Indeed, a phase II trial (NEJ013A trial) revealed the efficacy of carboplatin/pemetrexed plus bevacizumab regarding the control rate of malignant pleural effusion without pleurodesis at 8 weeks after treatment (92.9%; 95% CI, 77 to 99%)[244]. However, as it was a phase II trial, there are caveats regarding the results.

Adding bevacizumab to platinum-based therapy significantly increased the incidence of grade 3 or higher AEs such as proteinuria, hypertension, hemorrhagic events, neutropenia, febrile neutropenia (FN), and treatment-related

death[245-247]. One of the most important AEs is hemoptysis/pulmonary hemorrhage, and bevacizumab is contraindicated in cases involving a history of hemoptysis. In the AVF-0757g trial, hemoptysis/pulmonary hemorrhage of grade 1/2 occurred in 25.4% of patients in the carboplatin/paclitaxel plus bevacizumab group, grade 3 or more hemoptysis occurred in 9%, and treatment-related death occurred in 6%[248]. Because hemoptysis of grade 3 or more occurred in patients with squamous cell carcinoma (67%), patients with a tumor cavity region (83%), and patients with a central tumor location (100%), these factors are considered as risk factors. In the ECOG 4599 trial[21] and AVAiL trial[249], patients with squamous cell carcinoma were therefore excluded. In addition, attention must be paid to the development of FN related to molecular therapy targeted against angiogenesis. Treatment-related death due to FN occurred in 1.2% of subjects in the ECOG 4599 trial[21] and 0.2% in the AVAiL trial[249]. Although FN was not observed in any cases involving treatment-related death in the REVEL trial, FN was observed in 16% of patients in this trial[22].

3.1. Anti-VEGF antibody

By binding specifically to VEGF-A, anti-VEGF antibody inhibits the binding between VEGF-A and its receptors (VEGFR-1 and -2). Anti-VEGF antibody exerts an antitumor effect by suppressing tumor angiogenesis and enhancing antitumor action of anticancer drugs when combined with anticancer drugs, and by reducing the interstitial pressure, which leads to normalization of tumor blood vessels and to improvement of vascular permeability. Although TKIs directly act on cancer cells, anti-VEGF antibody acts on cancer cells indirectly.

3.1.1. Bevacizumab (AVASTIN®)

Bevacizumab is a monoclonal antibody that specifically binds to VEGF-A. A phase III trial of advanced non-squamous NSCLC patients (ECOG4599 trial) revealed a significant improvement in OS in the carboplatin/paclitaxel plus bevacizumab group compared to the carboplatin/paclitaxel group (HR for progression or death, 0.79; 95% CI, 0.67 to 0.92; P<0.003; 12.3 vs. 10.3 months)[21] (Table 6). Another phase III trial of chemo-naïve non-squamous NSCLC patients (AVAiL trial) also revealed a significant improvement in PFS, which was the primary endpoint of this study, in the cisplatin/gemcitabine plus 7.5 mg/kg bevacizumab group (HR, 0.75; 95% CI, 0.64 to 0.87; P=0.0003) and the cisplatin/gemcitabine plus 15 mg/kg bevacizumab group (HR, 0.85; 95% CI, 0.73 to 1.00; P=0.0456) compared to the cisplatin/gemcitabine group[249]. Furthermore, a phase III trial of advanced or recurrent non-squamous NSCLC patients (BEYOND trial) also revealed a significant improvement in PFS in the carboplatin/paclitaxel plus bevacizumab group compared to the carboplatin/paclitaxel group (HR, 0.40; 95% CI, 0.29 to 0.54; P<0.001; 9.2 vs. 6.5 months) and OS in the carboplatin/paclitaxel plus bevacizumab group compared to the carboplatin/paclitaxel group (HR, 0.68; 95% CI, 0.50 to 0.93; P=0.0154; 24.3 vs. 17.7 months)[250]. Regarding the administration period of bevacizumab, it is common to continue administration until disease progression or toxicity-related discontinuation after completion of platinum-based combination therapy[21, 249, 250].

Brain metastasis is a frequent complication of lung cancer. It lowers quality of life (QOL) and is associated with poor prognosis. The effect of cytocidal anticancer agents on brain metastasis is low, and radiotherapy is recommended for symptomatic brain metastasis. A phase II trial evaluating the safety of bevacizumab in non-squamous NSCLC patients with locally treated brain metastasis after a platinum doublet regimen or erlotinib (PASSPORT trial) revealed that cranial hemorrhage of grade 2 or more was obserbed[251]. Another phase II trial (BRAIN trial) evaluated the efficacy and safety of bevacizumab in non-squamous NSCLC patients with previously untreated brain metastasis[252]. In this trial, bevacizumab was added to first-line carboplatin/paclitaxel or second-line erlotinib treatment. The PFS was 6.7 months (95% CI, 5.7 to 7.1) and the OS reached 16 months. The response rate (RR) of intracranial legions was 61.2%. This RR was excellent, because the RR of intracranial legions to standard treatment (cisplatin plus pemetrexed) is 41%[253].

An initial regimen of chemotherapy used in combination with bevacizumab and the effectiveness of maintenance treatment with bevacizumab after the completion of initial chemotherapy were examined in the PointBreak trial[254], PRONOUNCE trial[255], and AVAPERL trial[256]. However, neither the PointBreak nor the PRONOUNCE trials improved on the results of the ECOG4599 trial, with both producing non-significant results. On the other hand, the AVAPERL trial, which evaluated the effectiveness of maintenance therapy with pemetrexed plus bevacizumab after cisplatin/pemetrexed plus bevacizumab, did not show statistical significance due to insufficient statistical power, although pemetrexed plus bevacizumab prolonged the OS for about 4 months.

Treatment involving continuing bevacizumab even after primary treatment PD is called bevacizumab beyond PD. A randomized phase II trial comparing docetaxel plus bevacizumab and docetaxel beyond PD after a platinum doublet regimen plus bevacizumab in advanced NSCLC patients (WJOG5910L trial) showed superiority of docetaxel plus bevacizumab regarding ORR, PFS, and OS[257]. A phase III trial confirming the efficacy of maintenance treatment with bevacizumab during any-line treatment beyond PD after a platinum doublet regimen plus bevacizumab (AvaALL trial) did not meet the primary endpoint, but it revealed that the OS tended to be better in the continuous bevacizumab group than in the standard therapy group (HR, 0.84; 90% CI, 0.71 to 1.00; P=0.1016; 11.9 vs. 10.2 months)[258].

3.2. Anti-VEGFR2 antibody

By binding to VEGFR-2, anti-VEGFR-2 antibody inhibits the binding not only of VEGF-A but also of VEGF-C and -D to their receptor (VEGFR-2). VEGF-C is associated with lymphangiogenesis, which has been reported to be associated with lymph node metastasis[259-262].

3.2.1. Ramucirumab (CYRAMZA®)

Ramucirumab is a human anti-VEGFR-2 monoclonal antibody that inhibits the proliferation, migration, and survival of endothelial cells by inhibition of activation of VEGFR-2 and it inhibits tumor angiogenesis. A phase III randomized controlled trial (REVEL trial) showed significant improvement in stage IV NSCLC patients who progressed during or after primary platinum-based chemotherapy[22]. The median OS was 10.5 months in the

docetaxel plus ramucirumab group versus 9.1 months in the docetaxel plus placebo group (HR: 0.857, 95% CI: 0.751-0.979, *P*=0.024). All grades of thrombocytopenia and grade 3/4 neutropenia and FN occurred more frequently in the docetaxel plus ramucirumab group. According to the subgroup analysis, the PFS (HR, 0.71; 95% CI, 0.57 to 0.88; *P*=0.002; 4.0 vs. 2.5 months) and ORR (22.5% vs. 12.6%) improved in the docetaxel plus ramucirumab group, even in patients refractory to primary therapy[263]. This trial has a very important significance in showing the effectiveness of docetaxel plus ramucirumab for NSCLC including squamous cell carcinoma.

4. IMMUNOTHERAPY/IMMUNO-CHECKPOINT BLOCKADE

T cells play a crucial role in cancer immunity, and mechanisms of regulation of T cell activation have been elucidated. In the regional lymph nodes, T cell receptors (TCRs) recognize antigens plus major histocompatibility complex (MHC) molecules expressed on dendritic cells, and the T cells are activated via this antigen presentation (priming phase). The T cells then migrate, infiltrate the tumor, and injure the tumor cells (effector phase)[264]. However, TCR signaling alone is insufficient for T cell activation and signaling from a co-stimulatory factor is necessary. In the priming phase, co-stimulatory factors include clusters of differentiation (CD) 28/CD80, CD28/CD86, CD40L/CD40, CD137/CD137L, OX40/OX40L, IL-2, and IL-12[265]. On the other hand, there are factors (co-inhibitory molecules) that prevent excessive activation and exhaustion of T cells, including CTLA-4/CD80, CTLA-4/CD86, PD-L1/PD-1, and prostaglandin. In the effector phase, the co-stimulatory factor is interferon (IFN)-γ, whereas the co-inhibitory molecules include PD-L1/PD-1, PD-L2/PD-1, indoleamine 2,3-dioxygenase (IDO), transforming growth factor (TGF)-β, V-domain Ig suppressor of T cell activation (VISTA), lymphocyte activation gene 3 (LAG-3), B- and T-lymphocyte attenuator (BTLA), B7-H3, MHC class I chain-related gene A (MICA)/MHC class I chain-related gene B (MICB), and T-cell immunoglobulin and mucin domain 3 (TIM3)/phospholipids[265]. These factors are called immune checkpoints and they control immunity[266].

In NSCLC, the RR to anti-PD-1/PD-L1 antibody monotherapy is only 20–30% (Table 7), but durable responses have been reported. Therefore predictive biomarkers need to be discovered. Expression of PD-L1 has been widely used as a predictive biomarker. Indeed, the KEYNOTE-001 trial showed that the RR to pembrolizumab increased according to the expression of PD-L1[267]. However, the expression of PD-L1 is heterogeneous even within the same tumor[268], and it changes with treatment progress and tumor development[269, 270]. Moreover, the measuring method and cutoff value of PD-L1 expression are different among pharmaceutical companies[271], and PD-L1 expression differs between fresh and preserved tissues[272, 273]. The "Blueprint PD-L1 IHC Assay Comparison Project" is an industry–academia collaboration project that provides information on analysis and clinical comparison of the four types of PD-L1 staining used in clinical trials (28-8, 22C3, SP142, and SP263). In this project, three experts independently evaluated the staining positivity rates. As a result, the proportion of PD-L1-positive tumor cells was similar for 28-8, 22C3, and SP263, whereas the proportion was lower for SP142[274]. Based on the expression cutoff values for each of the four types of PD-L1 staining, 50% (19/38) of cases were classified as at or above the cutoffs and 13% (5/38) as below the cutoffs. In the remaining cases (37%), the categorization of PD-L1 expression varied depending on which stain was used. More data are needed to determine the specific treatments to use according to PD-L1 staining cutoff values. The inconsistency rate of PD-L1 expression in lung cancer biopsy specimens and corresponding surgical specimens reached as high as 48%[275]. PD-L1 expression in lung squamous cell carcinoma in primary lesions and regional lymph nodes was consistent in 70.3% (52/74) of cases. The combination of primary tumor-negative and lymph node-positive results occurred in 10.8% (8/74) of cases and the combination of primary tumor-positive and lymph node-negative results occurred in 6.5% (5/77) of cases[276]. Further investigation is needed regarding the site of collection, timing, and methods[277]. There is a possibility that PD-L1 immunoreactivity is attenuated in specimen blocks created >5 years ago. Another promising predictive biomarker of ICI efficacy is tumor mutation burden (TMB)[278]. Mutation quantities in various types of cancer using 1200 tumor specimens assessed by whole exome sequencing (WES) have been reported[279]. Currently, mutation quantity is quantified as TMB. TMB is the number of somatic mutations in a tumor genome. TMB varies from tumor to tumor from 0.001 per megabase (Mb) to 400 per Mb. NSCLC has been reported to be a cancer with a relatively high TMB[280]. This is because the lung tissue is exposed to tobacco. Melanoma, involving skin tissues that are exposed to ultraviolet light, also has a high TMB. In the discovery cohort of NSCLC treated with pembrolizumab (n=16), high TMB (defined as above the median burden) was associated with better durable clinical benefit (DCB, partial or stable response lasting >6 months), ORR, and PFS than low TMB (below median) (73% vs. 13%, Fisher's exact *P*=0.04; 63% vs. 0%, Fisher's exact *P*=0.03; HR, 0.19; 95% CI, 0.05 to 0.70; 14.5 vs. 3.7 months, *P*=0.01, respectively)[278]. In the validation cohort of NSCLC treated with pembrolizumab (n=18), high TMB was also associated with better durable clinical benefit and PFS than low TMB (83% vs. 22%, Fisher's exact P=0.04; HR, 0.15; 95% CI, 0.04 to 0.59; not reached vs. 3.4 months, P=0.006, respectively)[278]. Another analysis of clinical annotation and response data from advanced NSCLC patients who received anti-PD-1 or anti-PD-L1 antibody (n=240) by targeted NGS also revealed that high TMB correlated well with sustained clinical benefit (38.6% vs. 25.1%, *P*=0.009). TMB is a variable that is independent of PD-L1 expression and predicts the benefit of an ICI[281]. Recent study demonstrated that high TMB was associated with better DCB, ORR, and PFS than low TMB (65% vs. 34%, Fisher's exact *P*=0.011; 51% vs. 13%, Fisher's exact *P*=0.0005; HR, 0.41; 95% CI, 0.23 to 0.73; 17.1 vs. 3.7 months, *P*=0.0024, respectively) in 75 NSCLC patients treated with combination immune checkpoint blockade[282]. Furthermore, multivariate analysis incorporating PD-L1 expression, histology, smoking status PS, and TMB revealed that TMB was independently associated with ORR (*P*=0.001) and PFS (*P*=0.002)[282]. Moreover, unbiased assessment of gene expression of tumor-infiltrating cells by single-cell RNA sequencing and longitudinal assessment of cellular protein expression by mass cytometry were currently

developed as novel markers and strategies that could stratify patients[283].

Immune checkpoints are involved in maintaining the homeostasis of immune responses and in the establishment of peripheral immune tolerance to self-antigens. Failure of this tolerance causes autoimmune diseases[284]. Related AEs are called immune-related adverse events (irAE). Although it is thought that T cells are the main immune cells involved in irAEs, B cells that produce antibodies and granulocytes that produce inflammatory cytokines are also thought to be involved[285-288]. The irAEs commonly occur in the skin, gastrointestinal tract (diarrhea and enteritis), liver, and endocrine system (hypothyroidism and type 1 diabetes mellitus, DM)[289]. But irAEs also occur in other parts of the body such as the kidneys, nerves (myasthenia gravis), muscles, and lungs (ILD). ILD is one of the most important irAEs and early detection is important. Paying attention to the initial symptoms such as fever, dry cough, and dyspnea, along with carrying out periodic chest X-ray photography and measurement of KL-6 and surfactant protein-D (SP-D), is necessary. ILD occurs in approximately 3% of cases involving anti-PD-1/PD-L1 antibody administration[290, 291]. ILD can involve cryptogenic organizing pneumonia (COP), nonspecific idiopathic pneumonia (NSIP), hypersensitivity pneumonitis (HP), and DAD[291]. In addition, peritumoral infiltration (PTI) is also a characteristic of ILD. Regarding the time of occurrence of irAEs, skin and gastrointestinal disorders appear early while hepatic and endocrine disorders often appear 1–2 months after the start of treatment. It should be noted that these disorders may appear 3–6 months after the start of treatment, so it is necessary to observe carefully for a long period[285]. Unlike symptomatic treatment during conventional cytotoxic chemotherapy, irAEs are treated with an immunosuppressant such as corticosteroids. In general, grade 1 irAE are treated symptomatically and ICI treatment is continued. In the case of grade 2 irAEs, symptomatic treatment is performed and suspension of ICI treatment until the irAEs resolve may be considered. In cases of prolonged and relapsed grade 2/3 irAEs, ICI treatment should be stopped and systemic administration of a corticosteroid may be considered. Although most irAEs can be treated by prompt and appropriate administration of a corticosteroid according to their severity, careful monitoring is necessary because severe or lethal cases can occur. If irAEs are poorly responsive to corticosteroid treatment, infliximab for enteritis, mycophenolate mofetil for liver injury, and intravenous immunoglobulin therapy for neuropathy should be considered[285, 292, 293].

The immune environment of the tumor varies greatly depending on the cancer type. In recent years, several genetic abnormalities that inhibit cancer immunity have been identified[294]. These genetic abnormalities include *TP53* loss-of-function mutation[295], *MYC*, *NOTCH2*, *FGFR1* amplification[296], *MYC* amplification[297], *PIK3CA* and *MET* mutations[296], *BRAF* mutations[298], *RAS* mutations[298], *VHL* and *STK11* mutations[296], and *NF1* loss-of-function [299]. Mutation of STK11 increases the production of IL-6, CXCL7, and granulocyte colony-stimulating factor (G-CSF) by cancer cells, thereby suppressing infiltration of CD8 T cells into cancer tissues due to effects on neutrophils, and the therapeutic effect is lost[300, 301]. Indeed, gene analysis of ipilimumab and nivolumab response cases indicated that wild-type STK11 was present in the response cases[282]. Interestingly, neutrophil infiltration is involved in resistance to the anti-PD-1 antibody[302].

4.1. Anti-PD-1 antibody

The PD-1 (CD279) molecule is an immunosuppressive auxiliary signal receptor belonging to the CD28 family and was first cloned in 1992 as a gene whose expression is induced by T cell death[303]. PD-1 is expressed in activated T and B cells, and when it binds to a ligand, it activates protein phosphatase in cells and suppresses antigen receptor signals[304]. As PD-1 is expressed in many cancer cells (such as kidney cancer, malignant melanoma, esophageal cancer, and ovarian cancer cells) and is related to the clinical course and prognosis, antibody drugs targeted to the PD-1 pathway have been developed[305, 306]. The anti-PD-1 antibody exerts an antitumor effect by blocking the PD-1/PD-L1 signal and preventing the suppression of effector T cells. Although CTLA-4-deficient mice develop a severe systemic inflammatory disease (irrespective of the mouse strain) and die a few weeks after birth, mice with a PD-1 signal dysfunction develop autoimmune diseases based on their genetic background[307]. C57B1/6 mice develop systemic lupus erythematosus (SLE)-like nephritis and arthritis[308], BALB/c mice develop dilated cardiomyopathy[309], and NOD mice develop type 1 DM[310]. These autoimmune diseases are also observed in humans.

4.1.1. Nivolumab (OPDIVO®)

Nivolumab is a fully human anti-PD-1 IgG4 monoclonal antibody. By binding to the extracellular region of PD-1, it inhibits the binding of PD-1 to PD-L1 and PD-L2 and enhances antigen-specific T cell activation. In other words, by enhancing T cell proliferation and IFN-γ production, it enhances the immune response to cancer and exerts an antitumor effect. Safety and efficacy (RR, 7.7%) were demonstrated by a phase I trial of nivolumab in 39 solid tumors.[311] The RRs in another phase I trial of nivolumab for a total 296 cases of NSCLC, melanoma, and renal cell carcinoma were 18%, 28% and 27%, respectively[312]. Frequent irAEs were rash (12%), diarrhea (11%), and pruritus (9%). Grade 3/4 irAEs were diarrhea (1%), liver dysfunction (1%), and ILD (1%) and 3 patients died of ILD. A phase III trial of nivolumab and docetaxel in previously treated squamous NSCLC patients (CheckMate 017 trial) revealed that nivolumab showed superiority regarding OS (HR, 0.59; 95% CI, 0.44 to 0.79; P<0.001; 9.2 vs. 6.0 months) and PFS (HR, 0.62; 95% CI, 0.47 to 0.81; P<0.001; 3.5 vs. 2.8 months)[313]. Furthermore, a phase III trial of nivolumab and docetaxel in previously treated non-squamous NSCLC patients (CheckMate 057 trial) also revealed that nivolumab showed superiority regarding OS (HR, 0.73; 95% CI, 0.59 to 0.89; P=0.002; 12.2 vs. 9.4 months), although the nivolumab group did not show superiority regarding PFS (HR, 0.92; 95% CI, 0.77 to 1.1; P=0.39; 2.3 vs. 4.2 months)[314]. The Kaplan-Meier curves showed that the nivolumab group exceeded the docetaxel group throughout the trial period in the CheckMate 017 trial, but in the CheckMate 057 trial, the Kaplan-Meier curve for the nivolumab group was initially below that for the docetaxel group and crossed over afterwards, suggesting that some cases of non-squamous NSCLC show no response to nivolumab at all. According to the immunohistochemically determined expression level of PD-L1 in the tumor tissues, subjects in both trials were divided into above and below

cutoff groups at cutoffs of 1%, 5% and 10%, and the OS was compared for each subgroup. In squamous NSCLC, the OS was significantly better in the nivolumab group than the docetaxel group regardless of PD-L1 expression. However, in non-squamous NSCLC with high PD-L1 expression, the OS tended to be better in the nivolumab group than the docetaxel group. In non-squamous NSCLC with low PD-L1 expression, there was no significant difference in the OS between the nivolumab and docetaxel groups.

On the other hand, a phase III trial comparing nivolumab and platinum-based chemotherapy in previously untreated NSCLC patients (CheckMate 026 trial) revealed that nivolumab did not show superiority regarding PFS (for patients with PD-L1 ≥5%), which was the primary endpoint of this trial (HR, 1.15; 95% CI, 0.91 to 1.45; *P*=0.25; 4.2 vs. 5.9 months)[315]. In addition, nivolumab (for patients with PD-L1 ≥5%) did not show superiority regarding OS (HR, 1.02; 95% CI, 0.80 to 1.31; 14.4 vs. 13.2 months) or ORR (odds ratio, 0.70; 95% CI, 0.46 to 1.06; 26 % vs. 33 %). However, in the analysis stratified by TMB, the PFS of high TMB patients tended to be better in the nivolumab group (HR, 0.62; 95% CI, 0.38 to 1.00; 9.7 vs. 5.8 months) while the PFS of the moderate-to-low TMB patients tended to be worse in the nivolumab group (HR, 1.82; 95% CI, 1.30 to 2.55; 4.1 vs. 6.9 months).

Together, these results indicate that nivolumab should be considered the standard second-line treatment for squamous cell carcinoma and a treatment option for non-squamous cell carcinoma.

4.1.2. Pembrolizumab (KEYTRUDA®)

Pembrolizumab is a human anti-PD-1 IgG4 monoclonal antibody. A phase III trial comparing 2 mg/kg pembrolizumab, 10 mg/kg pembrolizumab, and docetaxel in previously treated advanced or recurrent NSCLC patients (KEYNOTE-010 trial) revealed that pembrolizumab showed superiority regarding OS at 2 mg/kg (HR, 0.71; 95% CI, 0.58 to 0.88; *P*=0.0008; 10.4 vs. 8.5 months) and 10 mg/kg (HR, 0.61; 95% CI, 0.49 to 0.75; *P*<0.0001; 12.7 vs. 8.5 months)[316]. Pembrolizumab also showed superiority regarding PFS at 10 mg/kg (HR, 0.79; 95% CI, 0.66 to 0.94; *P*=0.004; 4.0 vs. 4.0 months) but not at 2 mg/kg (HR, 0.88; 95% CI, 0.74 to 1.05; *P*=0.07; 3.9 vs. 4.0 months). In patients with PD-L1 ≥50%, pembrolizumab showed superiority regarding OS at 2 mg/kg (HR, 0.54; *P*=0.0002; 14.9 vs. 8.2 months) and 10 mg/kg (HR, 0.50; *P*<0.0001; 17.3 vs. 8.2 months). Similarly, in patients with PD-L1 ≥50%, pembrolizumab showed superiority regarding PFS at 2 mg/kg (HR, 0.59; *P*=0.0001; 5.0 vs. 4.1 months) and 10 mg/kg (HR, 0.59; *P*<0.0001; 5.2 vs. 4.1 months). These results suggest that pembrolizumab is an effective agent in previously treated NSCLC, and PD-L1 is a biomarker of pembrolizumab efficacy. The reason why pembrolizumab showed superiority in the KEYNOTE-010 trial, whereas nivolumab did not show superiority in the CheckMate 026 trial is that the characteristics of the patients at baseline such as PD-L1 expression level and tumor mutation burden may have favored the chemotherapy group.

Furthermore, a phase III trial comparing pembrolizumab at a fixed dose of 200 mg every 3 weeks and platinum-based chemotherapy in untreated advanced NSCLC patients with PD-L1 ≥50% (KEYNOTE-024 trial) revealed that pembrolizumab showed superiority regarding PFS, which was the primary endpoint (HR, 0.50; 95% CI, 0.37 to 0.68; *P*<0.001; 10.3 vs. 6.0 months)[317], suggesting that pembrolizumab is also effective as a first-line treatment for NSCLC with PD-L1 ≥50%.

Studies on combination therapy involving ICI and chemotherapy have also been conducted. A phase III trial comparing platinum-based chemotherapy plus pembrolizumab and platinum-based chemotherapy plus placebo in untreated advanced non-squamous NSCLC patients (KEYNOTE-189 trial) revealed that platinum-based chemotherapy plus pembrolizumab showed superiority regarding OS at 12 months (HR, 0.52; 95% CI, 0.43 to 0.64; *P*<0.001; 69.2% vs. 46.4%) and PFS (HR, 0.50; 95% CI, 0.37 to 0.68; *P*<0.001; 8.8 vs. 4.9 months)[318]. In addition, a phase III trial comparing platinum-based chemotherapy plus pembrolizumab and platinum-based chemotherapy plus placebo in untreated advanced squamous NSCLC patients (KEYNOTE-407 trial) also revealed that platinum-based chemotherapy plus pembrolizumab showed superiority regarding OS (HR, 0.64; 95% CI, 0.49 to 0.85; *P*=0.0008; 15.9 vs. 11.3 months) and PFS (HR, 0.56; 95% CI, 0.45 to 0.70; *P*<0.0001; 6.4 vs. 4.8 months)[319]. Even in the subgroup analysis stratified by PD-L1 tumor proportion score (TPS), the PFS was superior in the combination group. Although the between-group difference in PFS for patients with TPS ≥50% was highly noticeable, it is noteworthy that the PFS was improved even for patients with TPS <1%. The frequency and severity of AEs were similar in both groups. Grade 3–5 AEs occurred in 69.8% of the patients in the combined group and 68.2% in the chemotherapy alone group, and treatment-related deaths occurred at a rate of 3.6% and 2.1%, respectively. AEs that resulted in discontinuation of all treatments occurred in 13.3% of the patients in the combined group and 6.4% of the patients in the chemotherapy alone group, and AEs that led to discontinuation of either treatment occurred at a rate of 23.4% and 11.8%, respectively. From the above results, it seems that combination therapy involving ICI and chemotherapy will become the standard therapy for initial treatment of NSCLC.

4.2. Anti-PD-L1 antibody

Two types of ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), have been identified as ligands for PD-1 and they are expressed on immune system cells such as dendritic cells, monocytes, and macrophages. PD-L1 is also expressed in non-immune organs such as the heart, lungs, liver, and placenta and it plays an important role in the maintenance of immune tolerance at the peripheral level[320]. On the other hand, various cancer cells and virus-infected cells also overexpress PD-L1, which is the cause of immune escape. The anti-PD-L1 antibody exerts its antitumor effect by blocking the PD-1/PD-L1 signal and releasing the suppression of effector T cells.

4.2.1. Atezolizumab (TECENTRIQ®)

Atezolizumab is a modified IgG1 monoclonal antibody against PD-L1. By substituting the amino acid at position 298 of the heavy chain from asparagine to alanine, the binding to Fc receptors is extremely impaired and antibody-dependent cellular cytotoxicity (ADCC) activity and complement-dependent cytotoxicity (CDC) activity are

eliminated. A phase II trial (POPLAR trial)[321] and a phase III trial (OAK trial)[322] comparing atezolizumab and docetaxel were conducted for second- and third-line treatment of advanced NSCLC regardless of the expression of PD-L1. The phase II trial of advanced non-squamous NSCLC patients (POPLAR trial) revealed a significant improvement of OS in the atezolizumab group compared to the docetaxel group (HR, 0.73; 95% CI, 0.53 to 0.99; *P*=0.04; 12.6 vs. 9.7 months)[321]. The subsequent phase III trial (OAK trial) showed the same results regarding the OS as in the phase II trial, that is, the OS for atezolizumab was significantly improved compared to that for docetaxel (HR, 0.73; 95% CI, 0.62 to 0.87; *P*=0.0003; 13.8 vs. 9.6 months)[322]. Neither trial included PD-L1 expression as an inclusion criterion, but it was used as a stratification factor. Expression of PD-L1 was immunohistochemically analyzed using SP142 antibody among tumor cells (TC) and tumor-infiltrating immune cells (IC). The cases were categorized into four subgroups according to the percentage of PD-L1-positive cells, designated from 0 to 3 for 0%; >0% but <5%; ≥5% but <50%; and ≥50%, respectively. Improvement in the OS due to atezolizumab was shown in all PD-L1 expression subgroups, but the HRs were 0.75, 0.74, 0.67, and 0.41 for TC0 and IC0, TC1/2/3 and IC1/2/3, TC2/3 and IC2/3, and TC3 and IC3, respectively. Intriguingly, even for patients in the TC0 and IC0 group, the OS for atezolizumab was significantly improved compared to that for docetaxel (HR, 0.75; 95% CI, 0.59 to 0.96; 12.6 vs. 8.9 months). Although this is the result of subgroup analysis and careful attention should be paid to the result, atezolizumab as well as nivolumab may be expected to be effective regardless of PD-L1 expression in previously treated advanced NSCLC patients. In the OAK trial, grade 3/4 AEs were found in 37% of the atezolizumab group and 54% of the docetaxel group. The AEs found in the atezolizumab group were fatigue, nausea, and loss of appetite. In the case of irAEs, diarrhea developed in 16%, hepatitis in 9%, hypothyroidism in 4%, and ILD in 2%.

In addition, a phase III trial (IMpower150 trial) was conducted to evaluate the effect of combination therapy involving atezolizumab and chemotherapy with bevacizumab as the primary treatment for advanced non-squamous NSCLC[323]. Subjects had non-squamous stage IV or recurrent NSCLC without chemotherapy treatment history and **t**here was no restriction based on PD-L1 expression level. The treatment arms were as follows: arm A was atezolizumab plus carboplatin/paclitaxel followed by atezolizumab maintenance, arm B was atezolizumab plus carboplatin/paclitaxel with bevacizumab followed by atezolizumab plus bevacizumab maintenance, and arm C was carboplatin/paclitaxel with bevacizumab followed by bevacizumab maintenance. The median OS of arm B in the intention to treat (ITT) analysis target group excluding cases involving *EGFR* or *ALK* mutations (ITT-WT), which was the main endpoint, was significantly improved compared to that in arm C (HR, 0.78; 95% CI, 0.64 to 0.96; *P*=0.0164; 19.2 vs. 14.7 months). On the basis of a predefined subgroup analysis, arm B was also superior to arm C for patients with any expression level of PD-L1, patients with *EGFR* or *ALK* mutations, and patients with liver metastasis. Grade 3/4 AEs occurred in 57% of patients in arm B and 49% in arm C, and grade 5 AEs occurred in 3% and 2%, respectively. As the primary treatment for advanced squamous NSCLC, a phase III trial (IMpower131 trial) was conducted to evaluate the effect of combination therapy involving atezolizumab and chemotherapy[324]. Subjects had stage IV squamous NSCLC without chemotherapy treatment history and **t**here was no restriction based on the PD-L1 expression level. The treatment arms were as follows: arm A was atezolizumab plus carboplatin/paclitaxel followed by atezolizumab maintenance, arm B was atezolizumab plus carboplatin/nab-paclitaxel followed by atezolizumab maintenance, and arm C was carboplatin/nab-paclitaxel. The median PFS in arm B was significantly improved compared to that in arm C (HR, 0.71; 95% CI, 0.60 to 0.85; *P*=0.0001; 6.3 vs. 5.6 months). Arm B was also superior to arm C for patients with any expression level of PD-L1. From the above results, it was suggested that combined use of ICI and chemotherapy is promising as a primary treatment.

4.2.2. Durvalumab (IMFINZI®)

Durvalumab is a modified IgG1 monoclonal antibody against PD-L1. By modifying Fc receptors, durvalumab eliminates ADCC activity and CDC activity. A phase III trial of locally advanced NSCLC patients (PACIFIC trial) revealed a significant improvement in PFS in the durvalumab after chemoradiotherapy group compared to the placebo after chemoradiotherapy group (HR, 0.52; 95% CI, 0.42 to 0.65; *P*<0.0001; 16.8 vs. 5.6 months)[325] (Table 3). Grade 3/4 AEs were found in 30% of patients in the durvalumab group and 26% in the placebo group. Any-grade pneumonitis/radiation pneumonitis was found in 34% of the durvalumab group and 25% of the placebo group. Grade 3/4 pneumonitis/radiation pneumonitis was found in 3% of both groups. Discontinuation of treatment occurred in 15% of the durvalumab group and 10% of the placebo group, mainly due to pneumonitis/radiation pneumonitis. However, treatment-related death occurred in 4% of cases in the durvalumab group and 6% in the placebo group. Therefore, durvalumab after chemoradiotherapy can be the standard treatment, although attention should be paid to the AEs, especially ILD.

4.2.3. Others

Avelumab is a fully human IgG1 antibody against PD-L1. A phase Ib trial of avelumab in previously treated NSCLC patients who were not selected by expression of PD-L1 (JAVELIN Solid Tumor trial) showed that the RR was 12% and the PFS was 11.6 weeks[326].

4.3. Anti-CTLA-4 antibody

CTLA-4 is expressed on activated T cells and transmits an inhibitory co-signal by binding to two B7 family molecules such as CD80 (B7-1) and CD86 (B7-2), which are expressed on APCs[327]. CD80/CD86 is also a ligand for the T cell co-stimulatory molecule CD28, but as the binding affinity of CD28 to CTLA-4 is tens of times higher than its binding affinity to CD80/CD86, CTLA-4 antagonistically inhibits the CD28 stimulatory co-signal. In addition, CTLA-4 activates protein phosphatase in cells and suppresses antigen receptor signals. The anti-CTLA-4 antibody ipilimumab promotes the proliferation and differentiation of antigen-specific effector cells by preventing the suppression of T cells in the priming phase. Furthermore, CTLA-4 is expressed constantly and at a high level in regulatory T cells, and has the function of suppressing APCs. Anti-CTLA-4 antibody not only inhibits the immunosuppressive function

of such regulatory T cells but also decreases regulatory T cells by ADCC or CDC activity. CTLA-4 has been known to play an important role in maintaining immune tolerance, because CTLA-4-deficient mice exhibit prominent proliferation of CD4-positive T cells and lethal autoimmune states[328, 329]. Indeed, in early clinical trials, irAEs such as colitis, ILD, and pituitary inflammation occurred in about 25–30% of the subjects.

4.3.1. Ipilimumab (YERVOY®)

Ipilimumab is an anti-CTLA-4 antibody and a major step in ICI development. Monotherapy with anti-CTLA-4 antibody in mice was not effective in a B16 melanoma mouse model with low immunogenicity, but it was effective in combination with a tumor vaccine, which led to the production of granulocyte macrophage colony-stimulating factor (GM-CSF), and immune memory was observed[330]. Therefore, for clinical trials in humans, ipilimumab and gp100 peptide vaccines were tested singly or in combination. Ipilimumab monotherapy improved the OS compared to the peptide vaccine monotherapy, but there was no synergistic effect with combined use of both drugs[331]. On the other hand, in a study in which high-dose ipilimumab and GM-CSF were used in combination, the combination was more effective than ipilimumab monotherapy[332]. Combined use of ipilimumab and a BRAF inhibitor was discontinued due to severe liver AEs[333]. No synergistic effect was observed with the combination of ipilimumab and high-dose IL-2[334], or the combination of ipilimumab and dacarbazine[335], and the RR was low. From the above results, some combinations of ipilimumab and other drugs show high effectiveness and some do not. A phase III trial was conducted to confirm the effect of combination therapy involving nivolumab and ipilimumab in patients with stage IV or recurrent untreated NSCLC without *EGFR* or *ALK* mutations (CheckMate 227)[336]. 1189 patients with PD-L1 expression ≥1% and 550 patients with PD-L1 expression <1% were randomly assigned to the nivolumab plus ipilimumab group, chemotherapy group, or nivolumab plus chemotherapy group. The PFS, which was the primary endpoint, of patients with high levels of TMB (≥10 mutations/Mb) was better in the nivolumab plus ipilimumab group than the chemotherapy group (HR, 0.48; 95% CI, 0.27 to 0.85; 7.7 vs. 5.3 months). The PFS of patients with high levels of TMB (≥10 mutations/Mb) was also better in the nivolumab plus chemotherapy group than the chemotherapy group (HR, 0.56; 95% CI, 0.35 to 0.91; 6.2 vs. 5.3 months). On the other hand, the PFS of patients with low levels of TMB (<10 mutations/Mb) was worse in the nivolumab plus chemotherapy group than the chemotherapy group (HR, 0.87; 95% CI, 0.57 to 1.33; 4.7 vs. 4.7 months). The PFS, which was the secondary endpoint, of patients with low expression levels of PD-L1 (<1%) was better in the nivolumab plus chemotherapy group than the chemotherapy group (HR, 0.74; 95% CI, 0.58 to 0.94; 5.6 vs. 4.7 months). Characteristics of patients with low expression levels of PD-L1 (<1%) were well-balanced between the nivolumab plus chemotherapy group and the chemotherapy group. Based on the above results, it is considered that combined use of ICI and chemotherapy and selection of patients by TMB will become standard strategies.

4.3.2. Others

Tremelimumab is a fully human IgG2 monoclonal antibody against CTLA-4[337]. A phase Ib trial of tremelimumab combined with durvalumab in immunotherapy-naïve NSCLC patients showed that the ORR was 23%[338].

5. FUTURE DIRECTIONS

5.1. Improvement of test equipment performance

NGS has advanced the diagnosis of driver oncogenes such as *HER2*, fibroblast growth factor receptor (*FGFR*), *NTRK1*, neuregulin 1 (*NRG1*), *BRAF*, and *MET*, and it is expected that the development of molecular targeted drugs will progress[339]. At ASCO 2018, the results of research verifying the cost of NGS and the cost of the other single-gene sequencing methods for new progressive NSCLC patients (N=2066) were reported[340]. In this study, NGS was used to simultaneously assess *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, *HER2*, *RET*, and *NTRK1*, which are eight genes involved in targeted NSCLC treatments. The sequence test is a test to assess one kind of gene at a time, the *KRAS* gene test is a test to exclude *KRAS* mutations, and the genetic panel test is a test to simultaneously assess *EGFR*, *ALK*, *ROS1*, and *BRAF* mutations. The total costs of testing the participants (N=2066) using the above four genetic tests was as follows: NGS, 2,190,499 dollars; sequence test, 3,721,368 dollars; *KRAS* gene test, 3,584,177 dollars; and gene panel test, 4,331,295 dollars. In addition, obtaining results for the sequence test took 2.8 weeks, the *KRAS* gene test took 2.7 weeks, and the gene panel test took 2 weeks, whereas NGS took 2 weeks. Thus, in the near future, if a new genetic mutation is identified, genetic testing that can identify multiple genetic mutations in a single test (such as NGS) may be more cost-effective than other methods.

On the other hand, the clinical importance of TMB is increasing in immunotherapy. Even though the analysis cost is becoming cheaper, TMB measurement by WES can only be carried out at specific facilities, so it is not widely available to everyone. Several studies have examined antitumor effect data along with TMB measurement using an NGS gene panel method in place of WES. The first study is a blood TMB (bTMB) measurement study that used blood specimens collected from patients participating in the POPLAR and OAK trials comparing atezolizumab and docetaxel for advanced NSCLC[341]. The bTMB was assessed using a 394-gene panel and the patients were classified into high and low groups, with 16/Mb as the cutoff value. The bTMB method was trialed in the POPLAR trial patients and then validated in the OAK trial patients. Atezolizumab was better than docetaxel regarding PFS for patients with bTMB ≥16/Mb (HR, 0.65; 95% CI, 0.47 to 0.92). On the other hand, atezolizumab did not show superiority regarding PFS for patients with bTMB <16/Mb (HR, 0.98; 95% CI, 0.80 to 1.20). Currently, prospective clinical trials are underway to stratify patients using bTMB. In another study, based on a TMB assay using a gene panel (MSK-IMPACT test) involving 341 (version 1), 410 (version 2), and 468 (version 3) genes developed by the Sloan Kettering Memorial Cancer Center, the correlation with the therapeutic effect of anti-PD-1 antibody or anti-PD-L1 antibody in 240 NSCLC patients was evaluated and compared with the correlation of TMB results assessed by the WES method[281]. These gene panels allow assessments of mutation in coding regions of 0.98 to

1.22 Mb. The mean TMB value based on these panels was 7.4 single nucleotide variations (SNVs)/Mb, and good response efficiency and PFS reproducibility were observed in high TMB patients. A comparison of TMB assessed by gene panel and TMB assessed by WES was carried out in 49 patients, and the TMB values of both methods were quite similar. From the above results, it is expected that TMB assessed by gene panel will be developed as a predictive marker of the treatment effect of ICIs.

5.2. Strategy to overcome resistance to TKI

To overcome resistance to molecular targeted therapy involving a bypass route (rather than secondary mutation), combination therapy that inhibits both the targeted molecule and the bypass route is thought to be important[342]. To overcome TKI tolerance, it is necessary to target pathways with different mechanisms of action that are not affected by TKIs. There is a report showing that Golgi function inhibitors are a promising treatment[343]. *EGFR* mutant lung cancer cells have been shown to develop a *BIM* gene polymorphism as a result of individual decreases in BIM protein expression that allowed them to become resistant to EGFR-TKI. It was also reported that tolerance could be removed by the additional use of a histone deacetylase inhibitor (vorinostat), which led to recovery of BIM protein expression[344, 345].

5.3. Improvement of postoperative prognosis using ICIs

The first-choice treatment for stage I/II NSCLC is lobectomy or pneumonectomy with lymph node excision[346]. However, postoperative radiotherapy did not improve survival after surgery but instead decreased survival[347]. In contrast, postoperative adjuvant chemotherapy using a cytotoxic anticancer drug slightly improved survival rate[348]. According to the Lung Adjuvant Cisplatin Evaluation (LACE) Collaborative Group, which carried out an pooled analysis, the additional effect of postoperative platinum-based chemotherapy on the 5-year OS was 5.4%[349]. Therefore, a novel postoperative adjuvant chemotherapy with low invasiveness that can be expected to improve prognosis is needed. Currently, ICI is attracting a great deal of interest and is being used for postoperative adjuvant therapy. One of the rationales of postoperative adjuvant therapy using ICI is that surgery may improve tumor-dependent immune suppression by significantly reducing the total amount of cancer cells[350]. On the other hand, it has been reported that the immune system is suppressed by surgical stress[351]. Several clinical trials have been started to date[352].

5.4. Positive effect of radiotherapy on tumor immune microenvironment (TIME)

Radiotherapy induces immunogenic cell death and enhances the release of tumor-associated antigens and damage associated molecular patterns (DAMPs). It also stimulates up-regulation of immune regulatory cell surface molecules and promotes the uptake of tumor antigens by dendritic cells, which cross-present tumor antigens to T cells, thereby triggering a cytotoxic T lymphocyte response[353-357]. In addition, regression of tumors outside of the radiation treatment field may be observed, which is known as the abscopal effect[358-363]. From the above results, synergistic effects can be expected between ICI and radiotherapy[364].

5.5. Possibility of combined use of a molecular targeted therapeutic agent and an ICI

As a result of a subgroup analysis of data from a large phase III trial, the effectiveness and safety of ICI for NSCLC with *EGFR* and *ALK* mutations are not supported[365]. This is partly due to the fact that the TMB of *EGFR* and *ALK* mutation-positive NSCLC is not high[366]. On the other hand, TMB is relatively high in *KRAS* and *BRAF* mutation-positive NSCLC. Furthermore, in preclinical studies, it has been reported that inhibition of the MAPK pathway improves host immunity by increasing the expression of melanoma antigen and by improving infiltration and function of T cells[367-373]. Indeed, in a phase I/II trial of melanoma patients, combination therapy involving ipilimumab and dabrafenib was effective[374]. The combination of a molecular targeted therapeutic agent and an ICI is a very attractive combination therapy. One of the candidate of this therapy is the anti-angiogenic metronomic chemotherapy[375]. Metronomic chemotherapy is a multi-targeted therapy and exerts both direct and indirect effects on tumor cells and TIME. It is expected to inhibit tumor angiogenesis and stimulate anticancer immune response[376].

LIST OF ABBREVIATIONS

ADCC	= antibody-dependent-cellular-cytotoxicity
AE	= adverse event
ALK	= anaplastic lymphoma kinase
APC	= antigen-presenting cell
ASCO	= American Society of Clinical Oncology
ARMS	= amplification refractory mutation system
BAC	= bronchiolar alveolar carcinoma
BRAF	= v-Raf murine sarcoma viral oncogene homolog B
BSC	= best supportive care
BTLA	= B- and T-lymphocyte attenuator
CCL	= C-C motif chemokine ligand
CD	= **c**lusters of differentiation
CDC	= complement-dependent cytotoxicity
cfDNA	= cell free DNA
CI	= confidence interval
CNS	= central nervous system
COP	= cryptogenic organizing pneumonia
CRKL	= CRK like proto-oncogene
CSF	= cerebrospinal fluid
CTCAE	= common terminology criteria for adverse events
CTL	= cytotoxic T lymphocyte
CTLA	= cytotoxic T-lymphocyte-associated protein
CXCL	= C-X-C motif chemokine
DAD	= diffuse alveolar damage
DAMP	= damage associated molecular pattern
DCB	= durable clinical benefit

DLT = dose-limiting toxicity

DM = diabetes mellitus

EGFR = epidermal growth factor receptor

EML4 = echinoderm microtubule-associated protein-like 4

EMT = epithelial-mesenchymal transition

FGFR = fibroblast growth factor receptor

FISH = fluorescence in situ hybridization

FN = febrile neutropenia

G-CSF = granulocyte colony-stimulating factor

GM-CSF = granulocyte macrophage colony-stimulating factor

HER = human epidermal growth factor receptor

HGF = hepatocyte growth factor

HP = hypersensitivity pneumonitis

HR = hazard ratio

HSP = heat shock protein

IASLC = International Association for the Study of Lung Cancer

IC = immune cell

ICI = immune checkpoint inhibitor

IDO = indoleamine 2,3-dioxygenase

IgG = immunoglobulin

IHC = immunohistochemistry

IFN-γ = interferon-γ

IL = interleukin

ILD = interstitial lung disease

ITT = intention to treat

irAE = immune-related adverse event

KIF5B = kinesin family 5B

LACE = Lung Adjuvant Cisplatin Evaluation

LAG-3 = lymphocyte activation gene 3

LAK = lymphokine-activated killer cell

MAPK = mitogen-activated protein kinase

Mb = megabase

MEK = mitogen-activated protein kinase kinase

MET = mesenchymal–epithelial transition factor;

MHC = major histocompatibility complex

MICA = MHC class I chain-related gene A

MICB = MHC class I chain-related gene B

MST = median survival time

MUC1 = mucin1

MVA = modified vaccinia Ankara

NGS = next-generation sequencer

NRG = neuregulin

NSCLC = non-small cell lung cancer

NSIP = nonspecific idiopathic pneumonia

NTRK = neurotropic tropomyosin receptor kinase

OR = odds ratio

ORR = overall response rate

OS = overall survival

PCR = polymerase chain reaction

PD = progressive disease

PD-1 = programmed cell death 1

PD-L1 = programmed death-ligand 1

PFS = progression-free survival

PI3K = phosphatidylinositol-3 kinase

PNA-LNA = peptide nucleic acid-locked nucleic acid

PTEN = phosphatase and tensin homolog

QOL = quality of life

RET = rearranged during transfection

ROS1 = c-ros oncogene 1

RR = response rate

RT = reverse transcription

SCLC = small cell lung cancer

SLE = systemic lupus erythematosus

SNV = single nucleotide variation

SP-D = surfactant protein-D

STAT = signal transducer and activator of t ranscription

TC = tumor cell

TCR = T cell receptor

TFG = TRK-fused gene

TGF-β = transforming growth factor-β

TIL = tumor infiltrating lymphocyte

TIME = tumor immune microenvironment

TIM3 = T-cell immunoglobulin and mucin domain 3

TKI = tyrosine kinase inhibitor

TMB = tumor mutation burden

TPS = tumor proportion score

TRK = tropomyosin receptor kinase

TTF-1 = thyroid transcription factor-1

VEGF = vascular endothelial growth factor

VEGFR = vascular endothelial growth factor receptor

VISTA = V-domain Ig suppressor of T cell activation

WES = whole exome sequencing

CONFLICT OF INTEREST

None declared.

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FIGURE LEGEND

Figure 1. The biochemical pathways in non-small cell lung cancer.

REFERENCES

[1] Minamoto, T.; Mai, M.; Ronai, Z., Environmental factors as regulators and effectors of multistep carcinogenesis. *Carcinogenesis* **1999,** *20* (4), 519-27.

[2] Pao, W.; Girard, N., New driver mutations in non-small-cell lung cancer. *Lancet Oncol* **2011,** *12* (2), 175-80.

[3] Wang, R.; Wang, L.; Li, Y.; Hu, H.; Shen, L.; Shen, X.; Pan, Y.; Ye, T.; Zhang, Y.; Luo, X.; Pan, B.; Li, B.; Li, H.; Zhang, J.; Pao, W.; Ji, H.; Sun, Y.; Chen, H., FGFR1/3 tyrosine kinase fusions define a unique molecular subtype of non-small cell lung cancer. *Clin Cancer Res* **2014,** *20* (15), 4107-14.

[4] Kim, H. S.; Park, K.; Jun, H. J.; Yi, S. Y.; Lee, J.; Ahn, J. S.; Park, Y. H.; Kim, S.; Lee, S.; Ahn, M. J., Comparison of survival in advanced non-small cell lung cancer patients in the pre- and post-gefitinib eras. *Oncology* **2009,** *76* (4), 239-46.

[5] Lynch, T. J.; Bell, D. W.; Sordella, R.; Gurubhagavatula, S.; Okimoto, R. A.; Brannigan, B. W.; Harris, P. L.; Haserlat, S. M.; Supko, J. G.; Haluska, F. G.; Louis, D. N.; Christiani, D. C.; Settleman, J.; Haber, D. A., Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* **2004,** *350* (21), 2129-39.

[6] Shepherd, F. A.; Rodrigues Pereira, J.; Ciuleanu, T.; Tan, E. H.; Hirsh, V.; Thongprasert, S.; Campos, D.; Maoleekoonpiroj, S.; Smylie, M.; Martins, R.; van Kooten, M.; Dediu, M.; Findlay, B.; Tu, D.; Johnston, D.; Bezjak, A.; Clark, G.; Santabárbara, P.; Seymour, L.; Group, N. C. I. o. C. C. T., Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* **2005,** *353* (2), 123-32.

[7] Park, K.; Tan, E. H.; O'Byrne, K.; Zhang, L.; Boyer, M.; Mok, T.; Hirsh, V.; Yang, J. C.; Lee, K. H.; Lu, S.; Shi, Y.; Kim, S. W.; Laskin, J.; Kim, D. W.; Arvis, C. D.; Kölbeck, K.; Laurie, S. A.; Tsai, C. M.; Shahidi, M.; Kim, M.; Massey, D.; Zazulina, V.; Paz-Ares, L., Afatinib versus gefitinib as first-line treatment of patients with EGFR mutation-positive non-small-cell lung cancer (LUX-Lung 7): a phase 2B, open-label, randomised controlled trial. *Lancet Oncol* **2016,** *17* (5), 577-89.

[8] Cross, D. A.; Ashton, S. E.; Ghiorghiu, S.; Eberlein, C.; Nebhan, C. A.; Spitzler, P. J.; Orme, J. P.; Finlay, M. R.; Ward, R. A.; Mellor, M. J.; Hughes, G.; Rahi, A.; Jacobs, V. N.; Red Brewer, M.; Ichihara, E.; Sun, J.; Jin, H.; Ballard, P.; Al-Kadhimi, K.; Rowlinson, R.; Klinowska, T.; Richmond, G. H.; Cantarini, M.; Kim, D. W.; Ranson, M. R.; Pao, W., AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. *Cancer Discov* **2014,** *4* (9), 1046-61.

[9] Soria, J. C.; Ohe, Y.; Vansteenkiste, J.; Reungwetwattana, T.; Chewaskulyong, B.; Lee, K. H.; Dechaphunkul, A.; Imamura, F.; Nogami, N.; Kurata, T.; Okamoto, I.; Zhou, C.; Cho, B. C.; Cheng, Y.; Cho, E. K.; Voon, P. J.; Planchard, D.; Su, W. C.; Gray, J. E.; Lee, S. M.; Hodge, R.; Marotti, M.; Rukazenkov, Y.; Ramalingam, S. S.; Investigators, F., Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. *N Engl J Med* **2018,** *378* (2), 113-125.

[10] Shaw, A. T.; Kim, D. W.; Nakagawa, K.; Seto, T.; Crinó, L.; Ahn, M. J.; De Pas, T.; Besse, B.; Solomon, B. J.; Blackhall, F.; Wu, Y. L.; Thomas, M.; O'Byrne, K. J.; Moro-Sibilot, D.; Camidge, D. R.; Mok, T.; Hirsh, V.; Riely, G. J.; Iyer, S.; Tassell, V.; Polli, A.; Wilner, K. D.; Jänne, P. A., Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med* **2013,** *368* (25), 2385-94.

[11] Bergethon, K.; Shaw, A. T.; Ou, S. H.; Katayama, R.; Lovly, C. M.; McDonald, N. T.; Massion, P. P.; Siwak-Tapp, C.; Gonzalez, A.; Fang, R.; Mark, E. J.; Batten, J. M.; Chen, H.; Wilner, K. D.; Kwak, E. L.; Clark, J. W.; Carbone, D. P.; Ji, H.; Engelman, J. A.; Mino-Kenudson, M.; Pao, W.; Iafrate, A. J., ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol* **2012,** *30* (8), 863-70.

[12] Kohno, T.; Tsuta, K.; Tsuchihara, K.; Nakaoku, T.; Yoh, K.; Goto, K., RET fusion gene: translation to personalized lung cancer therapy. *Cancer Sci* **2013,** *104* (11), 1396-400.

[13] Planchard, D.; Besse, B.; Groen, H. J. M.; Souquet, P. J.; Quoix, E.; Baik, C. S.; Barlesi, F.; Kim, T. M.; Mazieres, J.; Novello, S.; Rigas, J. R.; Upalawanna, A.; D'Amelio, A. M.; Zhang, P.; Mookerjee, B.; Johnson, B. E., Dabrafenib plus trametinib in patients with previously treated BRAF(V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. *Lancet Oncol* **2016,** *17* (7), 984-993.

[14] Peters, S.; Camidge, D. R.; Shaw, A. T.; Gadgeel, S.; Ahn, J. S.; Kim, D. W.; Ou, S. I.; Pérol, M.; Dziadziuszko, R.; Rosell, R.; Zeaiter, A.; Mitry, E.; Golding, S.; Balas, B.; Noe, J.; Morcos, P. N.; Mok, T.; Investigators, A. T., Alectinib versus Crizotinib in Untreated ALK-Positive Non-Small-Cell Lung Cancer. *N Engl J Med* **2017,** *377* (9), 829-838.

[15] Shaw, A. T.; Kim, D. W.; Mehra, R.; Tan, D. S.; Felip, E.; Chow, L. Q.; Camidge, D. R.; Vansteenkiste, J.; Sharma, S.; De Pas, T.; Riely, G. J.; Solomon, B. J.; Wolf, J.; Thomas, M.; Schuler, M.; Liu, G.; Santoro, A.; Lau, Y. Y.; Goldwasser, M.; Boral, A. L.; Engelman, J. A., Ceritinib in ALK-rearranged non-small-cell lung cancer. *N Engl J Med* **2014,** *370* (13), 1189-97.

[16] Shaw, A. T.; Friboulet, L.; Leshchiner, I.; Gainor, J. F.; Bergqvist, S.; Brooun, A.; Burke, B. J.; Deng, Y. L.; Liu, W.; Dardaei, L.; Frias, R. L.; Schultz, K. R.; Logan, J.; James, L. P.; Smeal, T.; Timofeevski, S.; Katayama, R.; Iafrate, A. J.; Le, L.; McTigue, M.; Getz, G.; Johnson, T. W.; Engelman, J. A., Resensitization to Crizotinib by the Lorlatinib ALK Resistance Mutation L1198F. *N Engl J Med* **2016,** *374* (1), 54-61.

[17] Brown, L. F.; Detmar, M.; Claffey, K.; Nagy, J. A.; Feng, D.; Dvorak, A. M.; Dvorak, H. F., Vascular permeability factor/vascular endothelial growth factor: a multifunctional angiogenic cytokine. *EXS* **1997,** *79*, 233-69.

[18] Dvorak, H. F.; Brown, L. F.; Detmar, M.; Dvorak, A. M., Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol* **1995,** *146* (5), 1029-39.

[19] Reinmuth, N.; Parikh, A. A.; Ahmad, S. A.; Liu, W.; Stoeltzing, O.; Fan, F.; Takeda, A.; Akagi, M.; Ellis, L. M., Biology of angiogenesis in tumors of the gastrointestinal tract. *Microsc Res Tech* **2003,** *60* (2), 199-207.

[20] Rafii, S.; Lyden, D.; Benezra, R.; Hattori, K.; Heissig, B., Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? *Nat Rev Cancer* **2002,** *2* (11), 826-35.

[21] Sandler, A.; Gray, R.; Perry, M. C.; Brahmer, J.; Schiller, J. H.; Dowlati, A.; Lilenbaum, R.; Johnson, D. H., Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* **2006,** *355* (24), 2542-50.

[22] Garon, E. B.; Ciuleanu, T. E.; Arrieta, O.; Prabhash, K.; Syrigos, K. N.; Goksel, T.; Park, K.; Gorbunova, V.; Kowalyszyn, R. D.; Pikiel, J.; Czyzewicz, G.; Orlov, S. V.; Lewanski, C. R.; Thomas, M.; Bidoli, P.; Dakhil, S.; Gans, S.; Kim, J. H.; Grigorescu, A.; Karaseva, N.; Reck, M.; Cappuzzo, F.; Alexandris, E.; Sashegyi, A.; Yurasov, S.; Pérol, M., Ramucirumab plus docetaxel versus placebo plus docetaxel for second-line treatment of stage IV non-small-cell lung cancer after disease progression on platinum-based therapy (REVEL): a multicentre, double-blind, randomised phase 3 trial. *Lancet* **2014,** *384* (9944), 665-73.

[23] Ribas, A., Releasing the Brakes on Cancer Immunotherapy. *N Engl J Med* **2015,** *373* (16), 1490-2.

[24] Walker, L. S.; Sansom, D. M., The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses. *Nat Rev Immunol* **2011,** *11* (12), 852-63.

[25] Teft, W. A.; Kirchhof, M. G.; Madrenas, J., A molecular perspective of CTLA-4 function. *Annu Rev Immunol* **2006,** *24*, 65-97.

[26] Forde, P. M.; Ettinger, D. S., Targeted therapy for non-small-cell lung cancer: past, present and future. *Expert Rev Anticancer Ther* **2013,** *13* (6), 745-58.

[27] Nascimento, A. V.; Bousbaa, H.; Ferreira, D.; Sarmento, B., Non-Small Cell Lung Carcinoma: An Overview on Targeted Therapy. *Curr Drug Targets* **2015,** *16* (13), 1448-63.

[28] Chan, B. A.; Hughes, B. G., Targeted therapy for non-small cell lung cancer: current standards and the promise of the future. *Transl Lung Cancer Res* **2015,** *4* (1), 36-54.

[29] Hirsch, F. R.; Jänne, P. A.; Eberhardt, W. E.; Cappuzzo, F.; Thatcher, N.; Pirker, R.; Choy, H.; Kim, E. S.; Paz-Ares, L.; Gandara, D. R.; Wu, Y. L.; Ahn, M. J.; Mitsudomi, T.; Shepherd, F. A.; Mok, T. S., Epidermal growth factor receptor inhibition in lung cancer: status 2012. *J Thorac Oncol* **2013,** *8* (3), 373-84.

[30] Paez, J. G.; Jänne, P. A.; Lee, J. C.; Tracy, S.; Greulich, H.; Gabriel, S.; Herman, P.; Kaye, F. J.; Lindeman, N.; Boggon, T. J.; Naoki, K.; Sasaki, H.; Fujii, Y.; Eck, M. J.; Sellers, W. R.; Johnson, B. E.; Meyerson, M., EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **2004,** *304* (5676), 1497-500.

[31] Mok, T. S.; Wu, Y. L.; Thongprasert, S.; Yang, C. H.; Chu, D. T.; Saijo, N.; Sunpaweravong, P.; Han, B.; Margono, B.; Ichinose, Y.; Nishiwaki, Y.; Ohe, Y.; Yang, J. J.; Chewaskulyong, B.; Jiang, H.; Duffield, E. L.; Watkins, C. L.; Armour, A. A.; Fukuoka, M., Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* **2009,** *361* (10), 947-57.

[32] Soda, M.; Choi, Y. L.; Enomoto, M.; Takada, S.; Yamashita, Y.; Ishikawa, S.; Fujiwara, S.; Watanabe, H.; Kurashina, K.; Hatanaka, H.; Bando, M.; Ohno, S.; Ishikawa, Y.; Aburatani, H.; Niki, T.; Sohara, Y.; Sugiyama, Y.; Mano, H., Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* **2007,** *448* (7153), 561-6.

[33] Shaw, A. T.; Ou, S. H.; Bang, Y. J.; Camidge, D. R.; Solomon, B. J.; Salgia, R.; Riely, G. J.; Varella-Garcia, M.; Shapiro, G. I.; Costa, D. B.; Doebele, R. C.; Le, L. P.; Zheng, Z.; Tan, W.; Stephenson, P.; Shreeve, S. M.; Tye, L. M.; Christensen, J. G.; Wilner, K. D.; Clark, J. W.; Iafrate, A. J., Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med* **2014,** *371* (21), 1963-71.

[34] Kris, M. G.; Johnson, B. E.; Berry, L. D.; Kwiatkowski, D. J.; Iafrate, A. J.; Wistuba, I. I.; Varella-Garcia, M.; Franklin, W. A.; Aronson, S. L.; Su, P. F.; Shyr, Y.; Camidge, D. R.; Sequist, L. V.; Glisson, B. S.; Khuri, F. R.; Garon, E. B.; Pao, W.; Rudin, C.; Schiller, J.; Haura, E. B.; Socinski, M.; Shirai, K.; Chen, H.; Giaccone, G.; Ladanyi, M.; Kugler, K.; Minna, J. D.; Bunn, P. A., Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. *JAMA* **2014,** *311* (19), 1998-2006.

[35] Mok, T. S.; Wu, Y. L.; Ahn, M. J.; Garassino, M. C.; Kim, H. R.; Ramalingam, S. S.; Shepherd, F. A.; He, Y.; Akamatsu, H.; Theelen, W. S.; Lee, C. K.; Sebastian, M.; Templeton, A.; Mann, H.; Marotti, M.;

Ghiorghiu, S.; Papadimitrakopoulou, V. A.; Investigators, A., Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer. *N Engl J Med* **2017,** *376* (7), 629-640.

[36] Choi, Y. L.; Soda, M.; Yamashita, Y.; Ueno, T.; Takashima, J.; Nakajima, T.; Yatabe, Y.; Takeuchi, K.; Hamada, T.; Haruta, H.; Ishikawa, Y.; Kimura, H.; Mitsudomi, T.; Tanio, Y.; Mano, H.; Group, A. L. C. S., EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *N Engl J Med* **2010,** *363* (18), 1734-9.

[37] Gainor, J. F.; Dardaei, L.; Yoda, S.; Friboulet, L.; Leshchiner, I.; Katayama, R.; Dagogo-Jack, I.; Gadgeel, S.; Schultz, K.; Singh, M.; Chin, E.; Parks, M.; Lee, D.; DiCecca, R. H.; Lockerman, E.; Huynh, T.; Logan, J.; Ritterhouse, L. L.; Le, L. P.; Muniappan, A.; Digumarthy, S.; Channick, C.; Keyes, C.; Getz, G.; Dias-Santagata, D.; Heist, R. S.; Lennerz, J.; Sequist, L. V.; Benes, C. H.; Iafrate, A. J.; Mino-Kenudson, M.; Engelman, J. A.; Shaw, A. T., Molecular Mechanisms of Resistance to First- and Second-Generation ALK Inhibitors in ALK-Rearranged Lung Cancer. *Cancer Discov* **2016,** *6* (10), 1118-1133.

[38] Hida, T.; Nokihara, H.; Kondo, M.; Kim, Y. H.; Azuma, K.; Seto, T.; Takiguchi, Y.; Nishio, M.; Yoshioka, H.; Imamura, F.; Hotta, K.; Watanabe, S.; Goto, K.; Satouchi, M.; Kozuki, T.; Shukuya, T.; Nakagawa, K.; Mitsudomi, T.; Yamamoto, N.; Asakawa, T.; Asabe, R.; Tanaka, T.; Tamura, T., Alectinib versus crizotinib in patients with ALK-positive non-small-cell lung cancer (J-ALEX): an open-label, randomised phase 3 trial. *Lancet* **2017,** *390* (10089), 29-39.

[39] Yarden, Y.; Sliwkowski, M. X., Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* **2001,** *2* (2), 127-37.

[40] Hynes, N. E.; Lane, H. A., ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat Rev Cancer* **2005,** *5* (5), 341-54.

[41] Yun, C. H.; Mengwasser, K. E.; Toms, A. V.; Woo, M. S.; Greulich, H.; Wong, K. K.; Meyerson, M.; Eck, M. J., The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc Natl Acad Sci U S A* **2008,** *105* (6), 2070-5.

[42] Akula, S.; Kamasani, S.; Sivan, S. K.; Manga, V.; Vudem, D. R.; Kancha, R. K., Computational Analysis of Epidermal Growth Factor Receptor Mutations Predicts Differential Drug Sensitivity Profiles toward Kinase Inhibitors. *J Thorac Oncol* **2018,** *13* (5), 721-726.

[43] Takano, T.; Ohe, Y.; Sakamoto, H.; Tsuta, K.; Matsuno, Y.; Tateishi, U.; Yamamoto, S.; Nokihara, H.; Yamamoto, N.; Sekine, I.; Kunitoh, H.; Shibata, T.; Sakiyama, T.; Yoshida, T.; Tamura, T., Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. *J Clin Oncol* **2005,** *23* (28), 6829-37.

[44] Kobayashi, S.; Canepa, H. M.; Bailey, A. S.; Nakayama, S.; Yamaguchi, N.; Goldstein, M. A.; Huberman, M. S.; Costa, D. B., Compound EGFR mutations and response to EGFR tyrosine kinase inhibitors. *J Thorac Oncol* **2013,** *8* (1), 45-51.

[45] Mitsudomi, T.; Kosaka, T.; Endoh, H.; Horio, Y.; Hida, T.; Mori, S.; Hatooka, S.; Shinoda, M.; Takahashi, T.; Yatabe, Y., Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. *J Clin Oncol* **2005,** *23* (11), 2513-20.

[46] Chan, S. K.; Gullick, W. J.; Hill, M. E., Mutations of the epidermal growth factor receptor in non-small cell lung cancer -- search and destroy. *Eur J Cancer* **2006,** *42* (1), 17-23.

[47] Kobayashi, S.; Boggon, T. J.; Dayaram, T.; Jänne, P. A.; Kocher, O.; Meyerson, M.; Johnson, B. E.; Eck, M. J.; Tenen, D. G.; Halmos, B., EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* **2005,** *352* (8), 786-92.

[48] Pao, W.; Miller, V. A.; Politi, K. A.; Riely, G. J.; Somwar, R.; Zakowski, M. F.; Kris, M. G.; Varmus, H., Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* **2005,** *2* (3), e73.

[49] Sheng, M.; Wang, F.; Zhao, Y.; Li, S.; Wang, X.; Shou, T.; Luo, Y.; Tang, W., Comparison of clinical outcomes of patients with non-small-cell lung cancer harbouring epidermal growth factor receptor exon 19 or exon 21 mutations after tyrosine kinase inhibitors treatment: a meta-analysis. *Eur J Clin Pharmacol* **2016,** *72* (1), 1-11.

[50] Eck, M. J.; Yun, C. H., Structural and mechanistic underpinnings of the differential drug sensitivity of EGFR mutations in non-small cell lung cancer. *Biochim Biophys Acta* **2010,** *1804* (3), 559-66.

[51] Carey, K. D.; Garton, A. J.; Romero, M. S.; Kahler, J.; Thomson, S.; Ross, S.; Park, F.; Haley, J. D.; Gibson, N.; Sliwkowski, M. X., Kinetic analysis of epidermal growth factor receptor somatic mutant proteins shows increased sensitivity to the epidermal growth factor receptor tyrosine kinase inhibitor, erlotinib. *Cancer Res* **2006,** *66* (16), 8163-71.

[52] Cho, J.; Chen, L.; Sangji, N.; Okabe, T.; Yonesaka, K.; Francis, J. M.; Flavin, R. J.; Johnson, W.; Kwon, J.; Yu, S.; Greulich, H.; Johnson, B. E.; Eck, M. J.; Jänne, P. A.; Wong, K. K.; Meyerson, M., Cetuximab response of lung cancer-derived EGF receptor mutants is associated with asymmetric dimerization. *Cancer Res* **2013,** *73* (22), 6770-9.

[53] Okabe, T.; Okamoto, I.; Tamura, K.; Terashima, M.; Yoshida, T.; Satoh, T.; Takada, M.; Fukuoka, M.; Nakagawa, K., Differential constitutive activation of the epidermal growth factor receptor in non-small cell lung cancer cells bearing EGFR gene mutation and amplification. *Cancer Res* **2007,** *67* (5), 2046-53.

[54] Oxnard, G. R.; Lo, P. C.; Nishino, M.; Dahlberg, S. E.; Lindeman, N. I.; Butaney, M.; Jackman, D. M.; Johnson, B. E.; Jänne, P. A., Natural history and molecular characteristics of lung cancers harboring EGFR exon 20 insertions. *J Thorac Oncol* **2013,** *8* (2), 179-84.

[55] Pan, Y.; Zhang, Y.; Li, Y.; Hu, H.; Wang, L.; Li, H.; Wang, R.; Ye, T.; Luo, X.; Li, B.; Cai, D.; Shen, L.; Sun, Y.; Chen, H., Prevalence, clinicopathologic characteristics, and molecular associations of EGFR exon 20 insertion mutations in East Asian patients with lung adenocarcinoma. *Ann Surg Oncol* **2014,** *21 Suppl 4*, S490-6.

[56] Beau-Faller, M.; Prim, N.; Ruppert, A. M.; Nanni-Metéllus, I.; Lacave, R.; Lacroix, L.; Escande, F.; Lizard, S.; Pretet, J. L.; Rouquette, I.; de Crémoux, P.; Solassol, J.; de Fraipont, F.; Bièche, I.; Cayre, A.; Favre-Guillevin, E.; Tomasini, P.; Wislez, M.; Besse, B.; Legrain, M.; Voegeli, A. C.; Baudrin, L.; Morin, F.; Zalcman, G.; Quoix, E.; Blons, H.; Cadranel, J., Rare EGFR exon 18 and exon 20 mutations in non-small-cell lung cancer on 10 117 patients: a multicentre observational study by the French ERMETIC-IFCT network. *Ann Oncol* **2014,** *25* (1), 126-31.

[57] Naidoo, J.; Sima, C. S.; Rodriguez, K.; Busby, N.; Nafa, K.; Ladanyi, M.; Riely, G. J.; Kris, M. G.; Arcila, M. E.; Yu, H. A., Epidermal growth factor receptor exon 20 insertions in advanced lung adenocarcinomas: Clinical outcomes and response to erlotinib. *Cancer* **2015,** *121* (18), 3212-3220.

[58] Yasuda, H.; Park, E.; Yun, C. H.; Sng, N. J.; Lucena-Araujo, A. R.; Yeo, W. L.; Huberman, M. S.; Cohen, D. W.; Nakayama, S.; Ishioka, K.; Yamaguchi, N.; Hanna, M.; Oxnard, G. R.; Lathan, C. S.; Moran, T.; Sequist, L. V.; Chaft, J. E.; Riely, G. J.; Arcila, M. E.; Soo, R. A.; Meyerson, M.; Eck, M. J.; Kobayashi, S. S.; Costa, D. B., Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer. *Sci Transl Med* **2013,** *5* (216), 216ra177.

[59] Voon, P. J.; Tsui, D. W.; Rosenfeld, N.; Chin, T. M., EGFR exon 20 insertion A763-Y764insFQEA and response to erlotinib--Letter. *Mol Cancer Ther* **2013,** *12* (11), 2614-5.

[60] Woo, H. S.; Ahn, H. K.; Lee, H. Y.; Park, I.; Kim, Y. S.; Hong, J.; Sym, S. J.; Park, J.; Lee, J. H.; Shin, D. B.; Cho, E. K., Epidermal growth factor receptor (EGFR) exon 20 mutations in non-small-cell lung cancer and resistance to EGFR-tyrosine kinase inhibitors. *Invest New Drugs* **2014,** *32* (6), 1311-5.

[61] Yasuda, H.; Kobayashi, S.; Costa, D. B., EGFR exon 20 insertion mutations in non-small-cell lung cancer: preclinical data and clinical implications. *Lancet Oncol* **2012,** *13* (1), e23-31.

[62] Yang, J. C.; Sequist, L. V.; Geater, S. L.; Tsai, C. M.; Mok, T. S.; Schuler, M.; Yamamoto, N.; Yu, C. J.; Ou, S. H.; Zhou, C.; Massey, D.; Zazulina, V.; Wu, Y. L., Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. *Lancet Oncol* **2015,** *16* (7), 830-8.

[63] Kim, E. Y.; Cho, E. N.; Park, H. S.; Hong, J. Y.; Lim, S.; Youn, J. P.; Hwang, S. Y.; Chang, Y. S., Compound EGFR mutation is frequently detected with co-mutations of actionable genes and associated with poor clinical outcome in lung adenocarcinoma. *Cancer Biol Ther* **2016,** *17* (3), 237-45.

[64] Hsu, F.; De Caluwe, A.; Anderson, D.; Nichol, A.; Toriumi, T.; Ho, C., Patterns of spread and prognostic implications of lung cancer metastasis in an era of driver mutations. *Curr Oncol* **2017,** *24* (4), 228-233.

[65] Jain, A.; Lim, C.; Gan, E. M.; Ng, D. Z.; Ng, Q. S.; Ang, M. K.; Takano, A.; Chan, K. S.; Tan, W. M.; Kanesvaran, R.; Toh, C. K.; Loo, C. M.; Hsu, A. A.; Devanand, A.; Lim, C. H.; Koong, H. N.; Koh, T.; Fong, K. W.; Yap, S. P.; Kim, S. W.; Chowbay, B.; Oon, L.; Lim, K. H.; Lim, W. T.; Tan, E. H.; Tan, D. S., Impact of Smoking and Brain Metastasis on Outcomes of Advanced EGFR Mutation Lung Adenocarcinoma Patients Treated with First Line Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors. *PLoS One* **2015,** *10* (5), e0123587.

[66] Kato, T.; Yoshioka, H.; Okamoto, I.; Yokoyama, A.; Hida, T.; Seto, T.; Kiura, K.; Massey, D.; Seki, Y.; Yamamoto, N., Afatinib versus cisplatin plus pemetrexed in Japanese patients with advanced non-small cell lung cancer harboring activating EGFR mutations: Subgroup analysis of LUX-Lung 3. *Cancer Sci* **2015,** *106* (9), 1202-11.

[67] Omuro, A. M.; Kris, M. G.; Miller, V. A.; Franceschi, E.; Shah, N.; Milton, D. T.; Abrey, L. E., High incidence of disease recurrence in the brain and leptomeninges in patients with nonsmall cell lung carcinoma after response to gefitinib. *Cancer* **2005,** *103* (11), 2344-8.

[68] Iuchi, T.; Shingyoji, M.; Sakaida, T.; Hatano, K.; Nagano, O.; Itakura, M.; Kageyama, H.; Yokoi, S.; Hasegawa, Y.; Kawasaki, K.; Iizasa, T., Phase II trial of gefitinib alone without radiation therapy for Japanese patients with brain metastases from EGFR-mutant lung adenocarcinoma. *Lung Cancer* **2013,** *82* (2), 282-7.

[69] Schuler, M.; Wu, Y. L.; Hirsh, V.; O'Byrne, K.; Yamamoto, N.; Mok, T.; Popat, S.; Sequist, L. V.; Massey, D.; Zazulina, V.; Yang, J. C., First-Line Afatinib versus Chemotherapy in Patients with Non-Small Cell Lung Cancer and Common Epidermal Growth Factor Receptor Gene Mutations and Brain Metastases. *J Thorac Oncol* **2016,** *11* (3), 380-90.

[70] Heon, S.; Yeap, B. Y.; Britt, G. J.; Costa, D. B.; Rabin, M. S.; Jackman, D. M.; Johnson, B. E., Development of central nervous system metastases in patients with advanced non-small cell lung cancer and somatic EGFR mutations treated with gefitinib or erlotinib. *Clin Cancer Res* **2010,** *16* (23), 5873-82.

[71] Togashi, Y.; Masago, K.; Fukudo, M.; Terada, T.; Fujita, S.; Irisa, K.; Sakamori, Y.; Kim, Y. H.; Mio, T.; Inui, K.; Mishima, M., Cerebrospinal fluid concentration of erlotinib and its active metabolite OSI-420 in patients with central nervous system metastases of non-small cell lung cancer. *J Thorac Oncol* **2010,** *5* (7), 950-5.

[72] Togashi, Y.; Masago, K.; Masuda, S.; Mizuno, T.; Fukudo, M.; Ikemi, Y.; Sakamori, Y.; Nagai, H.; Kim, Y. H.; Katsura, T.; Mishima, M., Cerebrospinal fluid concentration of gefitinib and erlotinib in patients

with non-small cell lung cancer. *Cancer Chemother Pharmacol* **2012,** *70* (3), 399-405.

[73] Tamiya, A.; Tamiya, M.; Nishihara, T.; Shiroyama, T.; Nakao, K.; Tsuji, T.; Takeuchi, N.; Isa, S. I.; Omachi, N.; Okamoto, N.; Suzuki, H.; Okishio, K.; Iwazaki, A.; Imai, K.; Hirashima, T.; Atagi, S., Cerebrospinal Fluid Penetration Rate and Efficacy of Afatinib in Patients with EGFR Mutation-positive Non-small Cell Lung Cancer with Leptomeningeal Carcinomatosis: A Multicenter Prospective Study. *Anticancer Res* **2017,** *37* (8), 4177-4182.

[74] Ballard, P.; Yates, J. W.; Yang, Z.; Kim, D. W.; Yang, J. C.; Cantarini, M.; Pickup, K.; Jordan, A.; Hickey, M.; Grist, M.; Box, M.; Johnström, P.; Varnäs, K.; Malmquist, J.; Thress, K. S.; Jänne, P. A.; Cross, D., Preclinical Comparison of Osimertinib with Other EGFR-TKIs in EGFR-Mutant NSCLC Brain Metastases Models, and Early Evidence of Clinical Brain Metastases Activity. *Clin Cancer Res* **2016,** *22* (20), 5130-5140.

[75] Nagai, Y.; Miyazawa, H.; Huqun; Tanaka, T.; Udagawa, K.; Kato, M.; Fukuyama, S.; Yokote, A.; Kobayashi, K.; Kanazawa, M.; Hagiwara, K., Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. *Cancer Res* **2005,** *65* (16), 7276-82.

[76] Kawada, I.; Soejima, K.; Watanabe, H.; Nakachi, I.; Yasuda, H.; Naoki, K.; Kawamura, M.; Eguchi, K.; Kobayashi, K.; Ishizaka, A., An alternative method for screening EGFR mutation using RFLP in non-small cell lung cancer patients. *J Thorac Oncol* **2008,** *3* (10), 1096-103.

[77] Endo, K.; Konishi, A.; Sasaki, H.; Takada, M.; Tanaka, H.; Okumura, M.; Kawahara, M.; Sugiura, H.; Kuwabara, Y.; Fukai, I.; Matsumura, A.; Yano, M.; Kobayashi, Y.; Mizuno, K.; Haneda, H.; Suzuki, E.; Iuchi, K.; Fujii, Y., Epidermal growth factor receptor gene mutation in non-small cell lung cancer using highly sensitive and fast TaqMan PCR assay. *Lung Cancer* **2005,** *50* (3), 375-84.

[78] Yatabe, Y.; Hida, T.; Horio, Y.; Kosaka, T.; Takahashi, T.; Mitsudomi, T., A rapid, sensitive assay to detect EGFR mutation in small biopsy specimens from lung cancer. *J Mol Diagn* **2006,** *8* (3), 335-41.

[79] Kimura, H.; Fujiwara, Y.; Sone, T.; Kunitoh, H.; Tamura, T.; Kasahara, K.; Nishio, K., High sensitivity detection of epidermal growth factor receptor mutations in the pleural effusion of non-small cell lung cancer patients. *Cancer Sci* **2006,** *97* (7), 642-8.

[80] Naoki, K.; Soejima, K.; Okamoto, H.; Hamamoto, J.; Hida, N.; Nakachi, I.; Yasuda, H.; Nakayama, S.; Yoda, S.; Satomi, R.; Ikemura, S.; Terai, H.; Sato, T.; Watanabe, K., The PCR-invader method (structure-specific 5' nuclease-based method), a sensitive method for detecting EGFR gene mutations in lung cancer specimens; comparison with direct sequencing. *Int J Clin Oncol* **2011,** *16* (4), 335-44.

[81] Kimura, H.; Ohira, T.; Uchida, O.; Matsubayashi, J.; Shimizu, S.; Nagao, T.; Ikeda, N.; Nishio, K., Analytical performance of the cobas EGFR mutation assay for Japanese non-small-cell lung cancer. *Lung Cancer* **2014,** *83* (3), 329-33.

[82] Marchetti, A.; Martella, C.; Felicioni, L.; Barassi, F.; Salvatore, S.; Chella, A.; Camplese, P. P.; Iarussi, T.; Mucilli, F.; Mezzetti, A.; Cuccurullo, F.; Sacco, R.; Buttitta, F., EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol* **2005,** *23* (4), 857-65.

[83] Sugio, K.; Uramoto, H.; Ono, K.; Oyama, T.; Hanagiri, T.; Sugaya, M.; Ichiki, Y.; So, T.; Nakata, S.; Morita, M.; Yasumoto, K., Mutations within the tyrosine kinase domain of EGFR gene specifically occur in lung adenocarcinoma patients with a low exposure of tobacco smoking. *Br J Cancer* **2006,** *94* (6), 896-903.

[84] Tsao, M. S.; Sakurada, A.; Ding, K.; Aviel-Ronen, S.; Ludkovski, O.; Liu, N.; Le Maître, A.; Gandara, D.; Johnson, D. H.; Rigas, J. R.; Seymour, L.; Shepherd, F. A., Prognostic and predictive value of epidermal growth factor receptor tyrosine kinase domain mutation status and gene copy number for adjuvant chemotherapy in non-small cell lung cancer. *J Thorac Oncol* **2011,** *6* (1), 139-47.

[85] Miyamae, Y.; Shimizu, K.; Hirato, J.; Araki, T.; Tanaka, K.; Ogawa, H.; Kakegawa, S.; Sugano, M.; Nakano, T.; Mitani, Y.; Kaira, K.; Takeyoshi, I., Significance of epidermal growth factor receptor gene mutations in squamous cell lung carcinoma. *Oncol Rep* **2011,** *25* (4), 921-8.

[86] Network, C. G. A. R., Comprehensive genomic characterization of squamous cell lung cancers. *Nature* **2012,** *489* (7417), 519-25.

[87] Pao, W.; Ladanyi, M., Epidermal growth factor receptor mutation testing in lung cancer: searching for the ideal method. *Clin Cancer Res* **2007,** *13* (17), 4954-5.

[88] Goto, K.; Satouchi, M.; Ishii, G.; Nishio, K.; Hagiwara, K.; Mitsudomi, T.; Whiteley, J.; Donald, E.; McCormack, R.; Todo, T., An evaluation study of EGFR mutation tests utilized for non-small-cell lung cancer in the diagnostic setting. *Ann Oncol* **2012,** *23* (11), 2914-9.

[89] Qiu, M.; Wang, J.; Xu, Y.; Ding, X.; Li, M.; Jiang, F.; Xu, L.; Yin, R., Circulating tumor DNA is effective for the detection of EGFR mutation in non-small cell lung cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* **2015,** *24* (1), 206-12.

[90] Tan, D. S.; Yom, S. S.; Tsao, M. S.; Pass, H. I.; Kelly, K.; Peled, N.; Yung, R. C.; Wistuba, I. I.; Yatabe, Y.; Unger, M.; Mack, P. C.; Wynes, M. W.; Mitsudomi, T.; Weder, W.; Yankelevitz, D.; Herbst, R. S.; Gandara, D. R.; Carbone, D. P.; Bunn, P. A.; Mok, T. S.; Hirsch, F. R., The International Association for the Study of Lung Cancer Consensus Statement on Optimizing Management of EGFR Mutation-Positive Non-Small Cell Lung Cancer: Status in 2016. *J Thorac Oncol* **2016,** *11* (7), 946-63.

[91] Reckamp, K. L.; Melnikova, V. O.; Karlovich, C.; Sequist, L. V.; Camidge, D. R.; Wakelee, H.; Perol, M.; Oxnard, G. R.; Kosco, K.; Croucher, P.; Samuelsz, E.; Vibat, C. R.; Guerrero, S.; Geis, J.; Berz, D.; Mann, E.; Matheny, S.; Rolfe, L.; Raponi, M.; Erlander, M. G.; Gadgeel, S., A Highly Sensitive and Quantitative Test Platform for Detection of NSCLC EGFR Mutations in Urine and Plasma. *J Thorac Oncol* **2016,** *11* (10), 1690-700.

[92] Pu, D.; Liang, H.; Wei, F.; Akin, D.; Feng, Z.; Yan, Q.; Li, Y.; Zhen, Y.; Xu, L.; Dong, G.; Wan, H.; Dong, J.; Qiu, X.; Qin, C.; Zhu, D.; Wang, X.; Sun, T.; Zhang, W.; Li, C.; Tang, X.; Qiao, Y.; Wong, D. T.; Zhou, Q., Evaluation of a novel saliva-based epidermal growth factor receptor mutation detection for lung cancer: A pilot study. *Thorac Cancer* **2016,** *7* (4), 428-36.

[93] Takeda, M.; Okamoto, I.; Nakagawa, K., Pooled safety analysis of EGFR-TKI treatment for EGFR mutation-positive non-small cell lung cancer. *Lung Cancer* **2015,** *88* (1), 74-9.

[94] Higenbottam, T.; Kuwano, K.; Nemery, B.; Fujita, Y., Understanding the mechanisms of drug-associated interstitial lung disease. *Br J Cancer* **2004,** *91 Suppl 2*, S31-7.

[95] Ando, M.; Okamoto, I.; Yamamoto, N.; Takeda, K.; Tamura, K.; Seto, T.; Ariyoshi, Y.; Fukuoka, M., Predictive factors for interstitial lung disease, antitumor response, and survival in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* **2006,** *24* (16), 2549-56.

[96] Hotta, K.; Kiura, K.; Tabata, M.; Harita, S.; Gemba, K.; Yonei, T.; Bessho, A.; Maeda, T.; Moritaka, T.; Shibayama, T.; Matsuo, K.; Kato, K.; Kanehiro, A.; Tanimoto, Y.; Ueoka, H.; Tanimoto, M., Interstitial lung disease in Japanese patients with non-small cell lung cancer receiving gefitinib: an analysis of risk factors and treatment outcomes in Okayama Lung Cancer Study Group. *Cancer J* **2005,** *11* (5), 417-24.

[97] Endo, M.; Johkoh, T.; Kimura, K.; Yamamoto, N., Imaging of gefitinib-related interstitial lung disease: multi-institutional analysis by the West Japan Thoracic Oncology Group. *Lung Cancer* **2006,** *52* (2), 135-40.

[98] Kashiwabara, K.; Semba, H.; Fujii, S.; Tsumura, S., Outcome in advanced non-small cell lung cancer patients with successful rechallenge after recovery from epidermal growth factor receptor tyrosine kinase inhibitor-induced interstitial lung disease. *Cancer Chemother Pharmacol* **2017,** *79* (4), 705-710.

[99] Kozuki, T., Skin problems and EGFR-tyrosine kinase inhibitor. *Jpn J Clin Oncol* **2016,** *46* (4), 291-8.

[100] Albanell, J.; Rojo, F.; Averbuch, S.; Feyereislova, A.; Mascaro, J. M.; Herbst, R.; LoRusso, P.; Rischin, D.; Sauleda, S.; Gee, J.; Nicholson, R. I.; Baselga, J., Pharmacodynamic studies of the epidermal growth factor receptor inhibitor ZD1839 in skin from cancer patients: histopathologic and molecular consequences of receptor inhibition. *J Clin Oncol* **2002,** *20* (1), 110-24.

[101] Baselga, J.; Rischin, D.; Ranson, M.; Calvert, H.; Raymond, E.; Kieback, D. G.; Kaye, S. B.; Gianni, L.; Harris, A.; Bjork, T.; Averbuch, S. D.; Feyereislova, A.; Swaisland, H.; Rojo, F.; Albanell, J., Phase I safety, pharmacokinetic, and pharmacodynamic trial of ZD1839, a selective oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with five selected solid tumor types. *J Clin Oncol* **2002,** *20* (21), 4292-302.

[102] Malik, S. N.; Siu, L. L.; Rowinsky, E. K.; deGraffenried, L.; Hammond, L. A.; Rizzo, J.; Bacus, S.; Brattain, M. G.; Kreisberg, J. I.; Hidalgo, M., Pharmacodynamic evaluation of the epidermal growth factor receptor inhibitor OSI-774 in human epidermis of cancer patients. *Clin Cancer Res* **2003,** *9* (7), 2478-86.

[103] Mascia, F.; Mariani, V.; Girolomoni, G.; Pastore, S., Blockade of the EGF receptor induces a deranged chemokine expression in keratinocytes leading to enhanced skin inflammation. *Am J Pathol* **2003,** *163* (1), 303-12.

[104] Rodeck, U.; Jost, M.; Kari, C.; Shih, D. T.; Lavker, R. M.; Ewert, D. L.; Jensen, P. J., EGF-R dependent regulation of keratinocyte survival. *J Cell Sci* **1997,** *110 (Pt 2)*, 113-21.

[105] Aw, D. C.; Tan, E. H.; Chin, T. M.; Lim, H. L.; Lee, H. Y.; Soo, R. A., Management of epidermal growth factor receptor tyrosine kinase inhibitor-related cutaneous and gastrointestinal toxicities. *Asia Pac J Clin Oncol* **2018,** *14* (1), 23-31.

[106] Hirsh, V., Managing treatment-related adverse events associated with egfr tyrosine kinase inhibitors in advanced non-small-cell lung cancer. *Curr Oncol* **2011,** *18* (3), 126-38.

[107] Uribe, J. M.; Gelbmann, C. M.; Traynor-Kaplan, A. E.; Barrett, K. E., Epidermal growth factor inhibits Ca(2+)-dependent Cl- transport in T84 human colonic epithelial cells. *Am J Physiol* **1996,** *271* (3 Pt 1), C914-22.

[108] Al-Dasooqi, N.; Gibson, R.; Bowen, J.; Keefe, D., HER2 targeted therapies for cancer and the gastrointestinal tract. *Curr Drug Targets* **2009,** *10* (6), 537-42.

[109] Yang, J. C.; Reguart, N.; Barinoff, J.; Köhler, J.; Uttenreuther-Fischer, M.; Stammberger, U.; O'Brien, D.; Wolf, J.; Cohen, E. E., Diarrhea associated with afatinib: an oral ErbB family blocker. *Expert Rev Anticancer Ther* **2013,** *13* (6), 729-36.

[110] Jänne, P. A.; Yang, J. C.; Kim, D. W.; Planchard, D.; Ohe, Y.; Ramalingam, S. S.; Ahn, M. J.; Kim, S. W.; Su, W. C.; Horn, L.; Haggstrom, D.; Felip, E.; Kim, J. H.; Frewer, P.; Cantarini, M.; Brown, K. H.; Dickinson, P. A.; Ghiorghiu, S.; Ranson, M., AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer. *N Engl J Med* **2015,** *372* (18), 1689-99.

[111] Thatcher, N.; Chang, A.; Parikh, P.; Rodrigues Pereira, J.; Ciuleanu, T.; von Pawel, J.; Thongprasert, S.; Tan, E. H.; Pemberton, K.; Archer, V.; Carroll, K., Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* **2005,** *366* (9496), 1527-37.

[112] Fukuoka, M.; Yano, S.; Giaccone, G.; Tamura, T.; Nakagawa, K.; Douillard, J. Y.; Nishiwaki, Y.; Vansteenkiste, J.; Kudoh, S.; Rischin, D.; Eek, R.; Horai, T.; Noda, K.; Takata, I.; Smit, E.; Averbuch, S.; Macleod, A.; Feyereislova, A.; Dong, R. P.; Baselga, J., Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. *J Clin Oncol* **2003,** *21* (12), 2237-46.

[113] Maruyama, R.; Nishiwaki, Y.; Tamura, T.; Yamamoto, N.; Tsuboi, M.; Nakagawa, K.; Shinkai, T.; Negoro, S.; Imamura, F.; Eguchi, K.; Takeda, K.; Inoue, A.; Tomii, K.; Harada, M.; Masuda, N.; Jiang, H.; Itoh, Y.; Ichinose, Y.; Saijo, N.; Fukuoka, M., Phase III study, V-15-32, of gefitinib versus docetaxel in previously treated Japanese patients with non-small-cell lung cancer. *J Clin Oncol* **2008,** *26* (26), 4244-52.

[114] Han, J. Y.; Park, K.; Kim, S. W.; Lee, D. H.; Kim, H. Y.; Kim, H. T.; Ahn, M. J.; Yun, T.; Ahn, J. S.; Suh, C.; Lee, J. S.; Yoon, S. J.; Han, J. H.; Lee, J. W.; Jo, S. J., First-SIGNAL: first-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. *J Clin Oncol* **2012,** *30* (10), 1122-8.

[115] Kim, E. S.; Hirsh, V.; Mok, T.; Socinski, M. A.; Gervais, R.; Wu, Y. L.; Li, L. Y.; Watkins, C. L.; Sellers, M. V.; Lowe, E. S.; Sun, Y.; Liao, M. L.; Osterlind, K.; Reck, M.; Armour, A. A.; Shepherd, F. A.; Lippman, S. M.; Douillard, J. Y., Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. *Lancet* **2008,** *372* (9652), 1809-18.

[116] Maemondo, M.; Inoue, A.; Kobayashi, K.; Sugawara, S.; Oizumi, S.; Isobe, H.; Gemma, A.; Harada, M.; Yoshizawa, H.; Kinoshita, I.; Fujita, Y.; Okinaga, S.; Hirano, H.; Yoshimori, K.; Harada, T.; Ogura, T.; Ando, M.; Miyazawa, H.; Tanaka, T.; Saijo, Y.; Hagiwara, K.; Morita, S.; Nukiwa, T.; Group, N.-E. J. S., Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* **2010,** *362* (25), 2380-8.

[117] Mitsudomi, T.; Morita, S.; Yatabe, Y.; Negoro, S.; Okamoto, I.; Tsurutani, J.; Seto, T.; Satouchi, M.; Tada, H.; Hirashima, T.; Asami, K.; Katakami, N.; Takada, M.; Yoshioka, H.; Shibata, K.; Kudoh, S.; Shimizu, E.; Saito, H.; Toyooka, S.; Nakagawa, K.; Fukuoka, M.; Group, W. J. O., Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* **2010,** *11* (2), 121-8.

[118] Chmielecki, J.; Foo, J.; Oxnard, G. R.; Hutchinson, K.; Ohashi, K.; Somwar, R.; Wang, L.; Amato, K. R.; Arcila, M.; Sos, M. L.; Socci, N. D.; Viale, A.; de Stanchina, E.; Ginsberg, M. S.; Thomas, R. K.; Kris, M. G.; Inoue, A.; Ladanyi, M.; Miller, V. A.; Michor, F.; Pao, W., Optimization of dosing for EGFR-mutant non-small cell lung cancer with evolutionary cancer modeling. *Sci Transl Med* **2011,** *3* (90), 90ra59.

[119] Soria, J. C.; Wu, Y. L.; Nakagawa, K.; Kim, S. W.; Yang, J. J.; Ahn, M. J.; Wang, J.; Yang, J. C.; Lu, Y.; Atagi, S.; Ponce, S.; Lee, D. H.; Liu, Y.; Yoh, K.; Zhou, J. Y.; Shi, X.; Webster, A.; Jiang, H.; Mok, T. S., Gefitinib plus chemotherapy versus placebo plus chemotherapy in EGFR-mutation-positive non-small-cell lung cancer after progression on first-line gefitinib (IMPRESS): a phase 3 randomised trial. *Lancet Oncol* **2015,** *16* (8), 990-8.

[120] Mok, T. S. K.; Kim, S. W.; Wu, Y. L.; Nakagawa, K.; Yang, J. J.; Ahn, M. J.; Wang, J.; Yang, J. C.; Lu, Y.; Atagi, S.; Ponce, S.; Shi, X.; Rukazenkov, Y.; Haddad, V.; Thress, K. S.; Soria, J. C., Gefitinib Plus Chemotherapy Versus Chemotherapy in Epidermal Growth Factor Receptor Mutation-Positive Non-Small-Cell Lung Cancer Resistant to First-Line Gefitinib (IMPRESS): Overall Survival and Biomarker Analyses. *J Clin Oncol* **2017,** *35* (36), 4027-4034.

[121] Oizumi, S.; Sugawara, S.; Minato, K.; Harada, T.; Inoue, A.; Fujita, Y.; Maemondo, M.; Watanabe, S.; Ito, K.; Gemma, A.; Demura, Y.; Fukumoto, S.; Isobe, H.; Kinoshita, I.; Morita, S.; Kobayashi, K.; Hagiwara, K.; Aiba, K.; Nukiwa, T., Updated survival outcomes of NEJ005/TCOG0902: a randomised phase II study of concurrent versus sequential alternating gefitinib and chemotherapy in previously untreated non-small cell lung cancer with sensitive. *ESMO Open* **2018,** *3* (2), e000313.

[122] Nakamura, A.; Inoue, A.; Morita, S.; Hosomi, Y.; Kato, T.; Fukuhara, T.; Gemma, A.; Takahashi, K.; Fujita, Y.; Harada, T.; Minato, K.; Takamura, K.; Kobayashi, K.; Nukiwa, T., Phase III study comparing gefitinib monotherapy (G) to combination therapy with gefitinib, carboplatin, and pemetrexed (GCP) for untreated patients (pts) with advanced non-small cell lung cancer (NSCLC) with EGFR mutations (NEJ009). **2018,** *36* (15_suppl), 9005-9005.

[123] Pérez-Soler, R.; Chachoua, A.; Hammond, L. A.; Rowinsky, E. K.; Huberman, M.; Karp, D.; Rigas, J.; Clark, G. M.; Santabárbara, P.; Bonomi, P., Determinants of tumor response and survival with erlotinib in patients with non--small-cell lung cancer. *J Clin Oncol* **2004,** *22* (16), 3238-47.

[124] Blackhall, F.; Ranson, M.; Thatcher, N., Where next for gefitinib in patients with lung cancer? *Lancet Oncol* **2006,** *7* (6), 499-507.

[125] Zhou, C.; Wu, Y. L.; Chen, G.; Feng, J.; Liu, X. Q.; Wang, C.; Zhang, S.; Wang, J.; Zhou, S.; Ren, S.; Lu, S.; Zhang, L.; Hu, C.; Luo, Y.; Chen, L.; Ye, M.; Huang, J.; Zhi, X.; Zhang, Y.; Xiu, Q.; Ma, J.; You, C., Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* **2011,** *12* (8), 735-42.

[126] Rosell, R.; Carcereny, E.; Gervais, R.; Vergnenegre, A.; Massuti, B.; Felip, E.; Palmero, R.; Garcia-Gomez, R.; Pallares, C.; Sanchez, J. M.; Porta, R.; Cobo, M.; Garrido, P.; Longo, F.; Moran, T.; Insa, A.; De Marinis, F.; Corre, R.; Bover, I.; Illiano, A.; Dansin, E.; de Castro, J.; Milella, M.; Reguart,

N.; Altavilla, G.; Jimenez, U.; Provencio, M.; Moreno, M. A.; Terrasa, J.; Muñoz-Langa, J.; Valdivia, J.; Isla, D.; Domine, M.; Molinier, O.; Mazieres, J.; Baize, N.; Garcia-Campelo, R.; Robinet, G.; Rodriguez-Abreu, D.; Lopez-Vivanco, G.; Gebbia, V.; Ferrera-Delgado, L.; Bombaron, P.; Bernabe, R.; Bearz, A.; Artal, A.; Cortesi, E.; Rolfo, C.; Sanchez-Ronco, M.; Drozdowskyj, A.; Queralt, C.; de Aguirre, I.; Ramirez, J. L.; Sanchez, J. J.; Molina, M. A.; Taron, M.; Paz-Ares, L.; Toracica, S. L. C. G. i. c. w. G. F. d. P.-C. a. A. I. O., Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* **2012,** *13* (3), 239-46.

[127] Urata, Y.; Katakami, N.; Morita, S.; Kaji, R.; Yoshioka, H.; Seto, T.; Satouchi, M.; Iwamoto, Y.; Kanehara, M.; Fujimoto, D.; Ikeda, N.; Murakami, H.; Daga, H.; Oguri, T.; Goto, I.; Imamura, F.; Sugawara, S.; Saka, H.; Nogami, N.; Negoro, S.; Nakagawa, K.; Nakanishi, Y., Randomized Phase III Study Comparing Gefitinib With Erlotinib in Patients With Previously Treated Advanced Lung Adenocarcinoma: WJOG 5108L. *J Clin Oncol* **2016,** *34* (27), 3248-57.

[128] Seto, T.; Kato, T.; Nishio, M.; Goto, K.; Atagi, S.; Hosomi, Y.; Yamamoto, N.; Hida, T.; Maemondo, M.; Nakagawa, K.; Nagase, S.; Okamoto, I.; Yamanaka, T.; Tajima, K.; Harada, R.; Fukuoka, M., Erlotinib alone or with bevacizumab as first-line therapy in patients with advanced non-squamous non-small-cell lung cancer harbouring EGFR mutations (JO25567): an open-label, randomised, multicentre, phase 2 study. *Lancet Oncol* **2014,** *15* (11), 1236-44.

[129] Furuya, N.; Fukuhara, T.; Saito, H.; Watanabe, K.; Sugawara, S.; Iwasawa, S.; Tsunezuka, Y.; Yamaguchi, O.; Okada, M.; Yoshimori, K.; Nakachi, I.; Gemma, A.; Azuma, K.; Hagiwara, K.; Nukiwa, T.; Morita, S.; Kobayashi, K.; Maemondo, M.; Group, N. E. J. S., Phase III study comparing bevacizumab plus erlotinib to erlotinib in patients with untreated NSCLC harboring activating EGFR mutations: NEJ026. *Journal of Clinical Oncology* **2018,** *36* (15_suppl), 9006-9006.

[130] Yamamoto, N.; Seto, T.; Nishio, M.; Goto, K.; Okamoto, I.; Yamanaka, T.; Tanaka, M.; Takahashi, K.; Fukuoka, M.; Yamamoto, N., Erlotinib plus bevacizumab (EB) versus erlotinib alone (E) as first-line treatment for advanced EGFR mutation–positive non-squamous non–small-cell lung cancer (NSCLC): Survival follow-up results of JO25567. *Journal of Clinical Oncology* **2018,** *36* (15_suppl), 9007-9007.

[131] Yang, J. C.; Shih, J. Y.; Su, W. C.; Hsia, T. C.; Tsai, C. M.; Ou, S. H.; Yu, C. J.; Chang, G. C.; Ho, C. L.; Sequist, L. V.; Dudek, A. Z.; Shahidi, M.; Cong, X. J.; Lorence, R. M.; Yang, P. C.; Miller, V. A., Afatinib for patients with lung adenocarcinoma and epidermal growth factor receptor mutations (LUX-Lung 2): a phase 2 trial. *Lancet Oncol* **2012,** *13* (5), 539-48.

[132] Sequist, L. V.; Yang, J. C.; Yamamoto, N.; O'Byrne, K.; Hirsh, V.; Mok, T.; Geater, S. L.; Orlov, S.; Tsai, C. M.; Boyer, M.; Su, W. C.; Bennouna, J.; Kato, T.; Gorbunova, V.; Lee, K. H.; Shah, R.; Massey, D.; Zazulina, V.; Shahidi, M.; Schduler, M., Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol* **2013,** *31* (27), 3327-34.

[133] Wu, Y. L.; Zhou, C.; Hu, C. P.; Feng, J.; Lu, S.; Huang, Y.; Li, W.; Hou, M.; Shi, J. H.; Lee, K. Y.; Xu, C. R.; Massey, D.; Kim, M.; Shi, Y.; Geater, S. L., Afatinib versus cisplatin plus gemcitabine for first-line treatment of Asian patients with advanced non-small-cell lung cancer harbouring EGFR mutations (LUX-Lung 6): an open-label, randomised phase 3 trial. *Lancet Oncol* **2014,** *15* (2), 213-22.

[134] Yang, J. C.; Wu, Y. L.; Schuler, M.; Sebastian, M.; Popat, S.; Yamamoto, N.; Zhou, C.; Hu, C. P.; O'Byrne, K.; Feng, J.; Lu, S.; Huang, Y.; Geater, S. L.; Lee, K. Y.; Tsai, C. M.; Gorbunova, V.; Hirsh, V.; Bennouna, J.; Orlov, S.; Mok, T.; Boyer, M.; Su, W. C.; Lee, K. H.; Kato, T.; Massey, D.; Shahidi, M.; Zazulina, V.; Sequist, L. V., Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials. *Lancet Oncol* **2015,** *16* (2), 141-51.

[135] Kobayashi, Y.; Mitsudomi, T., Not all epidermal growth factor receptor mutations in lung cancer are created equal: Perspectives for individualized treatment strategy. *Cancer Sci* **2016,** *107* (9), 1179-86.

[136] Nanjo, S.; Ebi, H.; Arai, S.; Takeuchi, S.; Yamada, T.; Mochizuki, S.; Okada, Y.; Nakada, M.; Murakami, T.; Yano, S., High efficacy of third generation EGFR inhibitor AZD9291 in a leptomeningeal carcinomatosis model with EGFR-mutant lung cancer cells. *Oncotarget* **2016,** *7* (4), 3847-56.

[137] Hirsh, V., Turning. *Ther Adv Med Oncol* **2018,** *10*, 1758834017753338.

[138] Mok, T. S.; Cheng, Y.; Zhou, X.; Lee, K. H.; Nakagawa, K.; Niho, S.; Lee, M.; Linke, R.; Rosell, R.; Corral, J.; Migliorino, M. R.; Pluzanski, A.; Sbar, E. I.; Wang, T.; White, J. L.; Wu, Y. L., Improvement in Overall Survival in a Randomized Study That Compared Dacomitinib With Gefitinib in Patients With Advanced Non-Small-Cell Lung Cancer and EGFR-Activating Mutations. *J Clin Oncol* **2018,** *36* (22), 2244-2250.

[139] Sequist, L. V.; Soria, J. C.; Goldman, J. W.; Wakelee, H. A.; Gadgeel, S. M.; Varga, A.; Papadimitrakopoulou, V.; Solomon, B. J.; Oxnard, G. R.; Dziadziuszko, R.; Aisner, D. L.; Doebele, R. C.; Galasso, C.; Garon, E. B.; Heist, R. S.; Logan, J.; Neal, J. W.; Mendenhall, M. A.; Nichols, S.; Piotrowska, Z.; Wozniak, A. J.; Raponi, M.; Karlovich, C. A.; Jaw-Tsai, S.; Isaacson, J.; Despain, D.; Matheny, S. L.; Rolfe, L.; Allen, A. R.; Camidge, D. R., Rociletinib in EGFR-mutated non-small-cell lung cancer. *N Engl J Med* **2015,** *372* (18), 1700-9.

[140] Thress, K. S.; Paweletz, C. P.; Felip, E.; Cho, B. C.; Stetson, D.; Dougherty, B.; Lai, Z.; Markovets, A.; Vivancos, A.; Kuang, Y.; Ercan, D.; Matthews, S. E.; Cantarini, M.; Barrett, J. C.; Jänne, P. A.; Oxnard, G. R., Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. *Nat Med* **2015,** *21* (6), 560-2.

[141] Ercan, D.; Choi, H. G.; Yun, C. H.; Capelletti, M.; Xie, T.; Eck, M. J.; Gray, N. S.; Jänne, P. A., EGFR Mutations and Resistance to Irreversible Pyrimidine-Based EGFR Inhibitors. *Clin Cancer Res* **2015,** *21* (17), 3913-23.

[142] Uchibori, K.; Inase, N.; Araki, M.; Kamada, M.; Sato, S.; Okuno, Y.; Fujita, N.; Katayama, R., Brigatinib combined with anti-EGFR antibody overcomes osimertinib resistance in EGFR-mutated non-small-cell lung cancer. *Nat Commun* **2017,** *8*, 14768.

[143] Planchard, D.; Loriot, Y.; André, F.; Gobert, A.; Auger, N.; Lacroix, L.; Soria, J. C., EGFR-independent mechanisms of acquired resistance to AZD9291 in EGFR T790M-positive NSCLC patients. *Ann Oncol* **2015,** *26* (10), 2073-8.

[144] Ho, C. C.; Liao, W. Y.; Lin, C. A.; Shih, J. Y.; Yu, C. J.; Chih-Hsin Yang, J., Acquired BRAF V600E Mutation as Resistant Mechanism after Treatment with Osimertinib. *J Thorac Oncol* **2017,** *12* (3), 567-572.

[145] Yano, S.; Wang, W.; Li, Q.; Matsumoto, K.; Sakurama, H.; Nakamura, T.; Ogino, H.; Kakiuchi, S.; Hanibuchi, M.; Nishioka, Y.; Uehara, H.; Mitsudomi, T.; Yatabe, Y.; Sone, S., Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. *Cancer Res* **2008,** *68* (22), 9479-87.

[146] Cheung, H. W.; Du, J.; Boehm, J. S.; He, F.; Weir, B. A.; Wang, X.; Butaney, M.; Sequist, L. V.; Luo, B.; Engelman, J. A.; Root, D. E.; Meyerson, M.; Golub, T. R.; Jänne, P. A.; Hahn, W. C., Amplification of CRKL induces transformation and epidermal growth factor receptor inhibitor resistance in human non-small cell lung cancers. *Cancer Discov* **2011,** *1* (7), 608-25.

[147] Sequist, L. V.; Waltman, B. A.; Dias-Santagata, D.; Digumarthy, S.; Turke, A. B.; Fidias, P.; Bergethon, K.; Shaw, A. T.; Gettinger, S.; Cosper, A. K.; Akhavanfard, S.; Heist, R. S.; Temel, J.; Christensen, J. G.; Wain, J. C.; Lynch, T. J.; Vernovsky, K.; Mark, E. J.; Lanuti, M.; Iafrate, A. J.; Mino-Kenudson, M.; Engelman, J. A., Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* **2011,** *3* (75), 75ra26.

[148] Ercan, D.; Xu, C.; Yanagita, M.; Monast, C. S.; Pratilas, C. A.; Montero, J.; Butaney, M.; Shimamura, T.; Sholl, L.; Ivanova, E. V.; Tadi, M.; Rogers, A.; Repellin, C.; Capelletti, M.; Maertens, O.; Goetz, E. M.; Letai, A.; Garraway, L. A.; Lazzara, M. J.; Rosen, N.; Gray, N. S.; Wong, K. K.; Jänne, P. A., Reactivation of ERK signaling causes resistance to EGFR kinase inhibitors. *Cancer Discov* **2012,** *2* (10), 934-47.

[149] Sos, M. L.; Koker, M.; Weir, B. A.; Heynck, S.; Rabinovsky, R.; Zander, T.; Seeger, J. M.; Weiss, J.; Fischer, F.; Frommolt, P.; Michel, K.; Peifer, M.; Mermel, C.; Girard, L.; Peyton, M.; Gazdar, A. F.; Minna, J. D.; Garraway, L. A.; Kashkar, H.; Pao, W.; Meyerson, M.; Thomas, R. K., PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. *Cancer Res* **2009,** *69* (8), 3256-61.

[150] Yamamoto, C.; Basaki, Y.; Kawahara, A.; Nakashima, K.; Kage, M.; Izumi, H.; Kohno, K.; Uramoto, H.; Yasumoto, K.; Kuwano, M.; Ono, M., Loss of PTEN expression by blocking nuclear translocation of EGR1 in gefitinib-resistant lung cancer cells harboring epidermal growth factor receptor-activating mutations. *Cancer Res* **2010,** *70* (21), 8715-25.

[151] Uramoto, H.; Iwata, T.; Onitsuka, T.; Shimokawa, H.; Hanagiri, T.; Oyama, T., Epithelial-mesenchymal transition in EGFR-TKI acquired resistant lung adenocarcinoma. *Anticancer Res* **2010,** *30* (7), 2513-7.

[152] Zhang, Z.; Lee, J. C.; Lin, L.; Olivas, V.; Au, V.; LaFramboise, T.; Abdel-Rahman, M.; Wang, X.; Levine, A. D.; Rho, J. K.; Choi, Y. J.; Choi, C. M.; Kim, S. W.; Jang, S. J.; Park, Y. S.; Kim, W. S.; Lee, D. H.; Lee, J. S.; Miller, V. A.; Arcila, M.; Ladanyi, M.; Moonsamy, P.; Sawyers, C.; Boggon, T. J.; Ma, P. C.; Costa, C.; Taron, M.; Rosell, R.; Halmos, B.; Bivona, T. G., Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. *Nat Genet* **2012,** *44* (8), 852-60.

[153] Huang, S.; Hölzel, M.; Knijnenburg, T.; Schlicker, A.; Roepman, P.; McDermott, U.; Garnett, M.; Grernrum, W.; Sun, C.; Prahallad, A.; Groenendijk, F. H.; Mittempergher, L.; Nijkamp, W.; Neefjes, J.; Salazar, R.; Ten Dijke, P.; Uramoto, H.; Tanaka, F.; Beijersbergen, R. L.; Wessels, L. F.; Bernards, R., MED12 controls the response to multiple cancer drugs through regulation of TGF-β receptor signaling. *Cell* **2012,** *151* (5), 937-50.

[154] Yao, Z.; Fenoglio, S.; Gao, D. C.; Camiolo, M.; Stiles, B.; Lindsted, T.; Schlederer, M.; Johns, C.; Altorki, N.; Mittal, V.; Kenner, L.; Sordella, R., TGF-beta IL-6 axis mediates selective and adaptive mechanisms of resistance to molecular targeted therapy in lung cancer. *Proc Natl Acad Sci U S A* **2010,** *107* (35), 15535-40.

[155] Shaw, A. T.; Yeap, B. Y.; Mino-Kenudson, M.; Digumarthy, S. R.; Costa, D. B.; Heist, R. S.; Solomon, B.; Stubbs, H.; Admane, S.; McDermott, U.; Settleman, J.; Kobayashi, S.; Mark, E. J.; Rodig, S. J.; Chirieac, L. R.; Kwak, E. L.; Lynch, T. J.; Iafrate, A. J., Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J Clin Oncol* **2009,** *27* (26), 4247-53.

[156] Lindeman, N. I.; Cagle, P. T.; Beasley, M. B.; Chitale, D. A.; Dacic, S.; Giaccone, G.; Jenkins, R. B.; Kwiatkowski, D. J.; Saldivar, J. S.; Squire, J.; Thunnissen, E.; Ladanyi, M., Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline

from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *J Thorac Oncol* **2013,** *8* (7), 823-59.

[157] Rikova, K.; Guo, A.; Zeng, Q.; Possemato, A.; Yu, J.; Haack, H.; Nardone, J.; Lee, K.; Reeves, C.; Li, Y.; Hu, Y.; Tan, Z.; Stokes, M.; Sullivan, L.; Mitchell, J.; Wetzel, R.; Macneill, J.; Ren, J. M.; Yuan, J.; Bakalarski, C. E.; Villen, J.; Kornhauser, J. M.; Smith, B.; Li, D.; Zhou, X.; Gygi, S. P.; Gu, T. L.; Polakiewicz, R. D.; Rush, J.; Comb, M. J., Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* **2007,** *131* (6), 1190-203.

[158] Takeuchi, K.; Choi, Y. L.; Togashi, Y.; Soda, M.; Hatano, S.; Inamura, K.; Takada, S.; Ueno, T.; Yamashita, Y.; Satoh, Y.; Okumura, S.; Nakagawa, K.; Ishikawa, Y.; Mano, H., KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. *Clin Cancer Res* **2009,** *15* (9), 3143-9.

[159] Inamura, K.; Takeuchi, K.; Togashi, Y.; Hatano, S.; Ninomiya, H.; Motoi, N.; Mun, M. Y.; Sakao, Y.; Okumura, S.; Nakagawa, K.; Soda, M.; Choi, Y. L.; Mano, H.; Ishikawa, Y., EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. *Mod Pathol* **2009,** *22* (4), 508-15.

[160] Rodig, S. J.; Mino-Kenudson, M.; Dacic, S.; Yeap, B. Y.; Shaw, A.; Barletta, J. A.; Stubbs, H.; Law, K.; Lindeman, N.; Mark, E.; Janne, P. A.; Lynch, T.; Johnson, B. E.; Iafrate, A. J.; Chirieac, L. R., Unique clinicopathologic features characterize ALK-rearranged lung adenocarcinoma in the western population. *Clin Cancer Res* **2009,** *15* (16), 5216-23.

[161] Katayama, R.; Shaw, A. T.; Khan, T. M.; Mino-Kenudson, M.; Solomon, B. J.; Halmos, B.; Jessop, N. A.; Wain, J. C.; Yeo, A. T.; Benes, C.; Drew, L.; Saeh, J. C.; Crosby, K.; Sequist, L. V.; Iafrate, A. J.; Engelman, J. A., Mechanisms of acquired crizotinib resistance in ALK-rearranged lung Cancers. *Sci Transl Med* **2012,** *4* (120), 120ra17.

[162] Gainor, J. F.; Shaw, A. T., Emerging paradigms in the development of resistance to tyrosine kinase inhibitors in lung cancer. *J Clin Oncol* **2013,** *31* (31), 3987-96.

[163] Toyokawa, G.; Seto, T., Updated Evidence on the Mechanisms of Resistance to ALK Inhibitors and Strategies to Overcome Such Resistance: Clinical and Preclinical Data. *Oncol Res Treat* **2015,** *38* (6), 291-8.

[164] Kodama, T.; Tsukaguchi, T.; Yoshida, M.; Kondoh, O.; Sakamoto, H., Selective ALK inhibitor alectinib with potent antitumor activity in models of crizotinib resistance. *Cancer Lett* **2014,** *351* (2), 215-21.

[165] Friboulet, L.; Li, N.; Katayama, R.; Lee, C. C.; Gainor, J. F.; Crystal, A. S.; Michellys, P. Y.; Awad, M. M.; Yanagitani, N.; Kim, S.; Pferdekamper, A. C.; Li, J.; Kasibhatla, S.; Sun, F.; Sun, X.; Hua, S.; McNamara, P.; Mahmood, S.; Lockerman, E. L.; Fujita, N.; Nishio, M.; Harris, J. L.; Shaw, A. T.; Engelman, J. A., The ALK inhibitor ceritinib overcomes crizotinib resistance in non-small cell lung cancer. *Cancer Discov* **2014,** *4* (6), 662-673.

[166] Katayama, R.; Khan, T. M.; Benes, C.; Lifshits, E.; Ebi, H.; Rivera, V. M.; Shakespeare, W. C.; Iafrate, A. J.; Engelman, J. A.; Shaw, A. T., Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. *Proc Natl Acad Sci U S A* **2011,** *108* (18), 7535-40.

[167] Katayama, R.; Friboulet, L.; Koike, S.; Lockerman, E. L.; Khan, T. M.; Gainor, J. F.; Iafrate, A. J.; Takeuchi, K.; Taiji, M.; Okuno, Y.; Fujita, N.; Engelman, J. A.; Shaw, A. T., Two novel ALK mutations mediate acquired resistance to the next-generation ALK inhibitor alectinib. *Clin Cancer Res* **2014,** *20* (22), 5686-96.

[168] Ignatius Ou, S. H.; Azada, M.; Hsiang, D. J.; Herman, J. M.; Kain, T. S.; Siwak-Tapp, C.; Casey, C.; He, J.; Ali, S. M.; Klempner, S. J.; Miller, V. A., Next-generation sequencing reveals a Novel NSCLC ALK F1174V mutation and confirms ALK G1202R mutation confers high-level resistance to alectinib (CH5424802/RO5424802) in ALK-rearranged NSCLC patients who progressed on crizotinib. *J Thorac Oncol* **2014,** *9* (4), 549-53.

[169] Park, S.; Park, T. S.; Choi, C. M.; Lee, D. H.; Kim, S. W.; Lee, J. S.; Kim, W. S.; Song, J. S.; Lee, J. C., Survival Benefit of Pemetrexed in Lung Adenocarcinoma Patients With Anaplastic Lymphoma Kinase Gene Rearrangements. *Clin Lung Cancer* **2015,** *16* (5), e83-9.

[170] Camidge, D. R.; Kono, S. A.; Lu, X.; Okuyama, S.; Barón, A. E.; Oton, A. B.; Davies, A. M.; Varella-Garcia, M.; Franklin, W.; Doebele, R. C., Anaplastic lymphoma kinase gene rearrangements in non-small cell lung cancer are associated with prolonged progression-free survival on pemetrexed. *J Thorac Oncol* **2011,** *6* (4), 774-80.

[171] Boland, J. M.; Erdogan, S.; Vasmatzis, G.; Yang, P.; Tillmans, L. S.; Johnson, M. R.; Wang, X.; Peterson, L. M.; Halling, K. C.; Oliveira, A. M.; Aubry, M. C.; Yi, E. S., Anaplastic lymphoma kinase immunoreactivity correlates with ALK gene rearrangement and transcriptional up-regulation in non-small cell lung carcinomas. *Hum Pathol* **2009,** *40* (8), 1152-8.

[172] Inamura, K.; Takeuchi, K.; Togashi, Y.; Nomura, K.; Ninomiya, H.; Okui, M.; Satoh, Y.; Okumura, S.; Nakagawa, K.; Soda, M.; Choi, Y. L.; Niki, T.; Mano, H.; Ishikawa, Y., EML4-ALK fusion is linked to histological characteristics in a subset of lung cancers. *J Thorac Oncol* **2008,** *3* (1), 13-7.

[173] Koivunen, J. P.; Mermel, C.; Zejnullahu, K.; Murphy, C.; Lifshits, E.; Holmes, A. J.; Choi, H. G.; Kim, J.; Chiang, D.; Thomas, R.; Lee, J.; Richards, W. G.; Sugarbaker, D. J.; Ducko, C.; Lindeman, N.; Marcoux, J. P.; Engelman, J. A.; Gray, N. S.; Lee, C.; Meyerson, M.; Jänne, P. A., EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. *Clin Cancer Res* **2008,** *14* (13), 4275-83.

[174] Salido, M.; Pijuan, L.; Martínez-Avilés, L.; Galván, A. B.; Cañadas, I.; Rovira, A.; Zanui, M.; Martínez, A.; Longarón, R.; Sole, F.; Serrano, S.; Bellosillo, B.; Wynes, M. W.; Albanell, J.; Hirsch, F. R.; Arriola, E., Increased ALK gene copy number and amplification are frequent in non-small cell lung cancer. *J Thorac Oncol* **2011,** *6* (1), 21-7.

[175] Takeuchi, K.; Choi, Y. L.; Soda, M.; Inamura, K.; Togashi, Y.; Hatano, S.; Enomoto, M.; Takada, S.; Yamashita, Y.; Satoh, Y.; Okumura, S.; Nakagawa, K.; Ishikawa, Y.; Mano, H., Multiplex reverse transcription-PCR screening for EML4-ALK fusion transcripts. *Clin Cancer Res* **2008,** *14* (20), 6618-24.

[176] Takahashi, T.; Sonobe, M.; Kobayashi, M.; Yoshizawa, A.; Menju, T.; Nakayama, E.; Mino, N.; Iwakiri, S.; Sato, K.; Miyahara, R.; Okubo, K.; Manabe, T.; Date, H., Clinicopathologic features of non-small-cell lung cancer with EML4-ALK fusion gene. *Ann Surg Oncol* **2010,** *17* (3), 889-97.

[177] Solomon, B. J.; Mok, T.; Kim, D. W.; Wu, Y. L.; Nakagawa, K.; Mekhail, T.; Felip, E.; Cappuzzo, F.; Paolini, J.; Usari, T.; Iyer, S.; Reisman, A.; Wilner, K. D.; Tursi, J.; Blackhall, F.; Investigators, P., First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med* **2014,** *371* (23), 2167-77.

[178] Camidge, D. R.; Kono, S. A.; Flacco, A.; Tan, A. C.; Doebele, R. C.; Zhou, Q.; Crino, L.; Franklin, W. A.; Varella-Garcia, M., Optimizing the detection of lung cancer patients harboring anaplastic lymphoma kinase (ALK) gene rearrangements potentially suitable for ALK inhibitor treatment. *Clin Cancer Res* **2010,** *16* (22), 5581-90.

[179] Chihara, D.; Suzuki, R., More on crizotinib. *N Engl J Med* **2011,** *364* (8), 776-7; author reply 778.

[180] Mino-Kenudson, M.; Chirieac, L. R.; Law, K.; Hornick, J. L.; Lindeman, N.; Mark, E. J.; Cohen, D. W.; Johnson, B. E.; Jänne, P. A.; Iafrate, A. J.; Rodig, S. J., A novel, highly sensitive antibody allows for the routine detection of ALK-rearranged lung adenocarcinomas by standard immunohistochemistry. *Clin Cancer Res* **2010,** *16* (5), 1561-71.

[181] Paik, J. H.; Choe, G.; Kim, H.; Choe, J. Y.; Lee, H. J.; Lee, C. T.; Lee, J. S.; Jheon, S.; Chung, J. H., Screening of anaplastic lymphoma kinase rearrangement by immunohistochemistry in non-small cell lung cancer: correlation with fluorescence in situ hybridization. *J Thorac Oncol* **2011,** *6* (3), 466-72.

[182] Zhu, Q.; Hu, H.; Weng, D. S.; Zhang, X. F.; Chen, C. L.; Zhou, Z. Q.; Tang, Y.; Xia, J. C., Pooled safety analyses of ALK-TKI inhibitor in ALK-positive NSCLC. *BMC Cancer* **2017,** *17* (1), 412.

[183] Soria, J. C.; Tan, D. S. W.; Chiari, R.; Wu, Y. L.; Paz-Ares, L.; Wolf, J.; Geater, S. L.; Orlov, S.; Cortinovis, D.; Yu, C. J.; Hochmair, M.; Cortot, A. B.; Tsai, C. M.; Moro-Sibilot, D.; Campelo, R. G.; McCulloch, T.; Sen, P.; Dugan, M.; Pantano, S.; Branle, F.; Massacesi, C.; de Castro, G., First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. *Lancet* **2017,** *389* (10072), 917-929.

[184] Kwak, E. L.; Bang, Y. J.; Camidge, D. R.; Shaw, A. T.; Solomon, B.; Maki, R. G.; Ou, S. H.; Dezube, B. J.; Jänne, P. A.; Costa, D. B.; Varella-Garcia, M.; Kim, W. H.; Lynch, T. J.; Fidias, P.; Stubbs, H.; Engelman, J. A.; Sequist, L. V.; Tan, W.; Gandhi, L.; Mino-Kenudson, M.; Wei, G. C.; Shreeve, S. M.; Ratain, M. J.; Settleman, J.; Christensen, J. G.; Haber, D. A.; Wilner, K.; Salgia, R.; Shapiro, G. I.; Clark, J. W.; Iafrate, A. J., Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* **2010,** *363* (18), 1693-703.

[185] Weickhardt, A. J.; Scheier, B.; Burke, J. M.; Gan, G.; Lu, X.; Bunn, P. A.; Aisner, D. L.; Gaspar, L. E.; Kavanagh, B. D.; Doebele, R. C.; Camidge, D. R., Local ablative therapy of oligoprogressive disease prolongs disease control by tyrosine kinase inhibitors in oncogene-addicted non-small-cell lung cancer. *J Thorac Oncol* **2012,** *7* (12), 1807-1814.

[186] Gan, G. N.; Weickhardt, A. J.; Scheier, B.; Doebele, R. C.; Gaspar, L. E.; Kavanagh, B. D.; Camidge, D. R., Stereotactic radiation therapy can safely and durably control sites of extra-central nervous system oligoprogressive disease in anaplastic lymphoma kinase-positive lung cancer patients receiving crizotinib. *Int J Radiat Oncol Biol Phys* **2014,** *88* (4), 892-8.

[187] Ou, S. H.; Jänne, P. A.; Bartlett, C. H.; Tang, Y.; Kim, D. W.; Otterson, G. A.; Crinò, L.; Selaru, P.; Cohen, D. P.; Clark, J. W.; Riely, G. J., Clinical benefit of continuing ALK inhibition with crizotinib beyond initial disease progression in patients with advanced ALK-positive NSCLC. *Ann Oncol* **2014,** *25* (2), 415-22.

[188] Tsuji, T.; Ozasa, H.; Aoki, W.; Aburaya, S.; Funazo, T.; Furugaki, K.; Yoshimura, Y.; Ajimizu, H.; Okutani, R.; Yasuda, Y.; Nomizo, T.; Uemasu, K.; Hasegawa, K.; Yoshida, H.; Yagi, Y.; Nagai, H.; Sakamori, Y.; Ueda, M.; Hirai, T.; Kim, Y. H., Alectinib Resistance in ALK-rearranged Lung Cancer by Dual Salvage Signaling in a Clinically Paired Resistance Model. *Mol Cancer Res* **2018**.

[189] Ou, S. H.; Ahn, J. S.; De Petris, L.; Govindan, R.; Yang, J. C.; Hughes, B.; Lena, H.; Moro-Sibilot, D.; Bearz, A.; Ramirez, S. V.; Mekhail, T.; Spira, A.; Bordogna, W.; Balas, B.; Morcos, P. N.; Monnet, A.; Zeaiter, A.; Kim, D. W., Alectinib in Crizotinib-Refractory ALK-Rearranged Non-Small-Cell Lung Cancer: A Phase II Global Study. *J Clin Oncol* **2016,** *34* (7), 661-8.

[190] Shaw, A. T.; Kim, T. M.; Crinò, L.; Gridelli, C.; Kiura, K.; Liu, G.; Novello, S.; Bearz, A.; Gautschi, O.; Mok, T.; Nishio, M.; Scagliotti, G.; Spigel, D. R.; Deudon, S.; Zheng, C.; Pantano, S.; Urban, P.; Massacesi, C.; Viraswami-Appanna, K.; Felip, E., Ceritinib versus chemotherapy in patients with ALK-rearranged non-small-cell lung cancer previously given chemotherapy and crizotinib (ASCEND-5): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol* **2017,** *18* (7), 874-886.

[191] Ou, S. H.; Klempner, S. J.; Greenbowe, J. R.; Azada, M.; Schrock, A. B.; Ali, S. M.; Ross, J. S.; Stephens, P. J.; Miller, V. A., Identification of a novel

HIP1-ALK fusion variant in Non-Small-Cell Lung Cancer (NSCLC) and discovery of ALK I1171 (I1171N/S) mutations in two ALK-rearranged NSCLC patients with resistance to Alectinib. *J Thorac Oncol* **2014,** *9* (12), 1821-5.

[192] Toyokawa, G.; Hirai, F.; Inamasu, E.; Yoshida, T.; Nosaki, K.; Takenaka, T.; Yamaguchi, M.; Seto, T.; Takenoyama, M.; Ichinose, Y., Secondary mutations at I1171 in the ALK gene confer resistance to both Crizotinib and Alectinib. *J Thorac Oncol* **2014,** *9* (12), e86-7.

[193] Ou, S. H.; Greenbowe, J.; Khan, Z. U.; Azada, M. C.; Ross, J. S.; Stevens, P. J.; Ali, S. M.; Miller, V. A.; Gitlitz, B., I1171 missense mutation (particularly I1171N) is a common resistance mutation in ALK-positive NSCLC patients who have progressive disease while on alectinib and is sensitive to ceritinib. *Lung Cancer* **2015,** *88* (2), 231-4.

[194] Hida, T.; Seto, T.; Horinouchi, H.; Maemondo, M.; Takeda, M.; Hotta, K.; Hirai, F.; Kim, Y. H.; Matsumoto, S.; Ito, M.; Ayukawa, K.; Tokushige, K.; Yonemura, M.; Mitsudomi, T.; Nishio, M., Phase II study of ceritinib in alectinib-pretreated patients with anaplastic lymphoma kinase-rearranged metastatic non-small-cell lung cancer in Japan: ASCEND-9. *Cancer Sci* **2018**.

[195] Gettinger, S. N.; Bazhenova, L. A.; Langer, C. J.; Salgia, R.; Gold, K. A.; Rosell, R.; Shaw, A. T.; Weiss, G. J.; Tugnait, M.; Narasimhan, N. I.; Dorer, D. J.; Kerstein, D.; Rivera, V. M.; Clackson, T.; Haluska, F. G.; Camidge, D. R., Activity and safety of brigatinib in ALK-rearranged non-small-cell lung cancer and other malignancies: a single-arm, open-label, phase 1/2 trial. *Lancet Oncol* **2016,** *17* (12), 1683-1696.

[196] Kim, D. W.; Tiseo, M.; Ahn, M. J.; Reckamp, K. L.; Hansen, K. H.; Kim, S. W.; Huber, R. M.; West, H. L.; Groen, H. J. M.; Hochmair, M. J.; Leighl, N. B.; Gettinger, S. N.; Langer, C. J.; Paz-Ares Rodríguez, L. G.; Smit, E. F.; Kim, E. S.; Reichmann, W.; Haluska, F. G.; Kerstein, D.; Camidge, D. R., Brigatinib in Patients With Crizotinib-Refractory Anaplastic Lymphoma Kinase-Positive Non-Small-Cell Lung Cancer: A Randomized, Multicenter Phase II Trial. *J Clin Oncol* **2017,** *35* (22), 2490-2498.

[197] Baglivo, S.; Ricciuti, B.; Ludovini, V.; Metro, G.; Siggillino, A.; De Giglio, A.; Chiari, R., Dramatic Response to Lorlatinib in a Heavily Pretreated Lung Adenocarcinoma Patient Harboring G1202R Mutation and a Synchronous Novel R1192P ALK Point Mutation. *J Thorac Oncol* **2018,** *13* (8), e145-e147.

[198] Shaw, A. T.; Felip, E.; Bauer, T. M.; Besse, B.; Navarro, A.; Postel-Vinay, S.; Gainor, J. F.; Johnson, M.; Dietrich, J.; James, L. P.; Clancy, J. S.; Chen, J.; Martini, J. F.; Abbattista, A.; Solomon, B. J., Lorlatinib in non-small-cell lung cancer with ALK or ROS1 rearrangement: an international, multicentre, open-label, single-arm first-in-man phase 1 trial. *Lancet Oncol* **2017,** *18* (12), 1590-1599.

[199] Drilon, A.; Siena, S.; Ou, S. I.; Patel, M.; Ahn, M. J.; Lee, J.; Bauer, T. M.; Farago, A. F.; Wheler, J. J.; Liu, S. V.; Doebele, R.; Giannetta, L.; Cerea, G.; Marrapese, G.; Schirru, M.; Amatu, A.; Bencardino, K.; Palmeri, L.; Sartore-Bianchi, A.; Vanzulli, A.; Cresta, S.; Damian, S.; Duca, M.; Ardini, E.; Li, G.; Christiansen, J.; Kowalski, K.; Johnson, A. D.; Patel, R.; Luo, D.; Chow-Maneval, E.; Hornby, Z.; Multani, P. S.; Shaw, A. T.; De Braud, F. G., Safety and Antitumor Activity of the Multitargeted Pan-TRK, ROS1, and ALK Inhibitor Entrectinib: Combined Results from Two Phase I Trials (ALKA-372-001 and STARTRK-1). *Cancer Discov* **2017,** *7* (4), 400-409.

[200] Horn, L.; Infante, J. R.; Reckamp, K. L.; Blumenschein, G. R.; Leal, T. A.; Waqar, S. N.; Gitlitz, B. J.; Sanborn, R. E.; Whisenant, J. G.; Du, L.; Neal, J. W.; Gockerman, J. P.; Dukart, G.; Harrow, K.; Liang, C.; Gibbons, J. J.; Holzhausen, A.; Lovly, C. M.; Wakelee, H. A., Ensartinib (X-396) in ALK-Positive Non-Small Cell Lung Cancer: Results from a First-in-Human Phase I/II, Multicenter Study. *Clin Cancer Res* **2018,** *24* (12), 2771-2779.

[201] Sequist, L. V.; Gettinger, S.; Senzer, N. N.; Martins, R. G.; Jänne, P. A.; Lilenbaum, R.; Gray, J. E.; Iafrate, A. J.; Katayama, R.; Hafeez, N.; Sweeney, J.; Walker, J. R.; Fritz, C.; Ross, R. W.; Grayzel, D.; Engelman, J. A.; Borger, D. R.; Paez, G.; Natale, R., Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. *J Clin Oncol* **2010,** *28* (33), 4953-60.

[202] Socinski, M. A.; Goldman, J.; El-Hariry, I.; Koczywas, M.; Vukovic, V.; Horn, L.; Paschold, E.; Salgia, R.; West, H.; Sequist, L. V.; Bonomi, P.; Brahmer, J.; Chen, L. C.; Sandler, A.; Belani, C. P.; Webb, T.; Harper, H.; Huberman, M.; Ramalingam, S.; Wong, K. K.; Teofilovici, F.; Guo, W.; Shapiro, G. I., A multicenter phase II study of ganetespib monotherapy in patients with genotypically defined advanced non-small cell lung cancer. *Clin Cancer Res* **2013,** *19* (11), 3068-77.

[203] Felip, E.; Barlesi, F.; Besse, B.; Chu, Q.; Gandhi, L.; Kim, S. W.; Carcereny, E.; Sequist, L. V.; Brunsvig, P.; Chouaid, C.; Smit, E. F.; Groen, H. J. M.; Kim, D. W.; Park, K.; Avsar, E.; Szpakowski, S.; Akimov, M.; Garon, E. B., Phase 2 Study of the HSP-90 Inhibitor AUY922 in Previously Treated and Molecularly Defined Patients with Advanced Non-Small Cell Lung Cancer. *J Thorac Oncol* **2018,** *13* (4), 576-584.

[204] Gainor, J. F.; Shaw, A. T., Novel targets in non-small cell lung cancer: ROS1 and RET fusions. *Oncologist* **2013,** *18* (7), 865-75.

[205] Goto, K.; Yang, J. C.-H.; Kim, D.-W.; Lu, S.; Seto, T.; Yang, J.-J.; Yamamoto, N.; Ahn, M.-J.; Takahashi, T.; Yamanaka, T.; Kemner, A. M.; Roychowdhury, D.; Paolini, J.; Wilner, K. D.; Wu, Y.-L., Phase II study of crizotinib in east Asian patients (pts) with ROS1-positive advanced non-small cell lung cancer (NSCLC). **2016,** *34* (15_suppl), 9022-9022.

[206] Awad, M. M.; Katayama, R.; McTigue, M.; Liu, W.; Deng, Y. L.; Brooun, A.; Friboulet, L.; Huang, D.; Falk, M. D.; Timofeevski, S.; Wilner, K. D.; Lockerman, E. L.; Khan, T. M.; Mahmood, S.; Gainor,

J. F.; Digumarthy, S. R.; Stone, J. R.; Mino-Kenudson, M.; Christensen, J. G.; Iafrate, A. J.; Engelman, J. A.; Shaw, A. T., Acquired resistance to crizotinib from a mutation in CD74-ROS1. *N Engl J Med* **2013,** *368* (25), 2395-401.

[207] Song, A.; Kim, T. M.; Kim, D. W.; Kim, S.; Keam, B.; Lee, S. H.; Heo, D. S., Molecular Changes Associated with Acquired Resistance to Crizotinib in ROS1-Rearranged Non-Small Cell Lung Cancer. *Clin Cancer Res* **2015,** *21* (10), 2379-87.

[208] Drilon, A.; Somwar, R.; Wagner, J. P.; Vellore, N. A.; Eide, C. A.; Zabriskie, M. S.; Arcila, M. E.; Hechtman, J. F.; Wang, L.; Smith, R. S.; Kris, M. G.; Riely, G. J.; Druker, B. J.; O'Hare, T.; Ladanyi, M.; Davare, M. A., A Novel Crizotinib-Resistant Solvent-Front Mutation Responsive to Cabozantinib Therapy in a Patient with ROS1-Rearranged Lung Cancer. *Clin Cancer Res* **2016,** *22* (10), 2351-8.

[209] Facchinetti, F.; Loriot, Y.; Kuo, M. S.; Mahjoubi, L.; Lacroix, L.; Planchard, D.; Besse, B.; Farace, F.; Auger, N.; Remon, J.; Scoazec, J. Y.; André, F.; Soria, J. C.; Friboulet, L., Crizotinib-Resistant ROS1 Mutations Reveal a Predictive Kinase Inhibitor Sensitivity Model for ROS1- and ALK-Rearranged Lung Cancers. *Clin Cancer Res* **2016,** *22* (24), 5983-5991.

[210] Marchetti, A.; Felicioni, L.; Malatesta, S.; Grazia Sciarrotta, M.; Guetti, L.; Chella, A.; Viola, P.; Pullara, C.; Mucilli, F.; Buttitta, F., Clinical features and outcome of patients with non-small-cell lung cancer harboring BRAF mutations. *J Clin Oncol* **2011,** *29* (26), 3574-9.

[211] Cardarella, S.; Ogino, A.; Nishino, M.; Butaney, M.; Shen, J.; Lydon, C.; Yeap, B. Y.; Sholl, L. M.; Johnson, B. E.; Jänne, P. A., Clinical, pathologic, and biologic features associated with BRAF mutations in non-small cell lung cancer. *Clin Cancer Res* **2013,** *19* (16), 4532-40.

[212] Falchook, G. S.; Long, G. V.; Kurzrock, R.; Kim, K. B.; Arkenau, T. H.; Brown, M. P.; Hamid, O.; Infante, J. R.; Millward, M.; Pavlick, A. C.; O'Day, S. J.; Blackman, S. C.; Curtis, C. M.; Lebowitz, P.; Ma, B.; Ouellet, D.; Kefford, R. F., Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: a phase 1 dose-escalation trial. *Lancet* **2012,** *379* (9829), 1893-901.

[213] Planchard, D.; Kim, T. M.; Mazieres, J.; Quoix, E.; Riely, G.; Barlesi, F.; Souquet, P. J.; Smit, E. F.; Groen, H. J.; Kelly, R. J.; Cho, B. C.; Socinski, M. A.; Pandite, L.; Nase, C.; Ma, B.; D'Amelio, A.; Mookerjee, B.; Curtis, C. M.; Johnson, B. E., Dabrafenib in patients with BRAF(V600E)-positive advanced non-small-cell lung cancer: a single-arm, multicentre, open-label, phase 2 trial. *Lancet Oncol* **2016,** *17* (5), 642-50.

[214] Flaherty, K. T.; Infante, J. R.; Daud, A.; Gonzalez, R.; Kefford, R. F.; Sosman, J.; Hamid, O.; Schuchter, L.; Cebon, J.; Ibrahim, N.; Kudchadkar, R.; Burris, H. A.; Falchook, G.; Algazi, A.; Lewis, K.; Long, G. V.; Puzanov, I.; Lebowitz, P.; Singh, A.; Little, S.; Sun, P.; Allred, A.; Ouellet, D.; Kim, K. B.; Patel, K.; Weber, J., Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N Engl J Med* **2012,** *367* (18), 1694-703.

[215] Larkin, J.; Ascierto, P. A.; Dréno, B.; Atkinson, V.; Liszkay, G.; Maio, M.; Mandalà, M.; Demidov, L.; Stroyakovskiy, D.; Thomas, L.; de la Cruz-Merino, L.; Dutriaux, C.; Garbe, C.; Sovak, M. A.; Chang, I.; Choong, N.; Hack, S. P.; McArthur, G. A.; Ribas, A., Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. *N Engl J Med* **2014,** *371* (20), 1867-76.

[216] Long, G. V.; Stroyakovskiy, D.; Gogas, H.; Levchenko, E.; de Braud, F.; Larkin, J.; Garbe, C.; Jouary, T.; Hauschild, A.; Grob, J. J.; Chiarion-Sileni, V.; Lebbe, C.; Mandalà, M.; Millward, M.; Arance, A.; Bondarenko, I.; Haanen, J. B.; Hansson, J.; Utikal, J.; Ferraresi, V.; Kovalenko, N.; Mohr, P.; Probachai, V.; Schadendorf, D.; Nathan, P.; Robert, C.; Ribas, A.; DeMarini, D. J.; Irani, J. G.; Swann, S.; Legos, J. J.; Jin, F.; Mookerjee, B.; Flaherty, K., Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial. *Lancet* **2015,** *386* (9992), 444-51.

[217] Mulligan, L. M., RET revisited: expanding the oncogenic portfolio. *Nat Rev Cancer* **2014,** *14* (3), 173-86.

[218] Takeuchi, K.; Soda, M.; Togashi, Y.; Suzuki, R.; Sakata, S.; Hatano, S.; Asaka, R.; Hamanaka, W.; Ninomiya, H.; Uehara, H.; Lim Choi, Y.; Satoh, Y.; Okumura, S.; Nakagawa, K.; Mano, H.; Ishikawa, Y., RET, ROS1 and ALK fusions in lung cancer. *Nat Med* **2012,** *18* (3), 378-81.

[219] Lipson, D.; Capelletti, M.; Yelensky, R.; Otto, G.; Parker, A.; Jarosz, M.; Curran, J. A.; Balasubramanian, S.; Bloom, T.; Brennan, K. W.; Donahue, A.; Downing, S. R.; Frampton, G. M.; Garcia, L.; Juhn, F.; Mitchell, K. C.; White, E.; White, J.; Zwirko, Z.; Peretz, T.; Nechushtan, H.; Soussan-Gutman, L.; Kim, J.; Sasaki, H.; Kim, H. R.; Park, S. I.; Ercan, D.; Sheehan, C. E.; Ross, J. S.; Cronin, M. T.; Jänne, P. A.; Stephens, P. J., Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. *Nat Med* **2012,** *18* (3), 382-4.

[220] Kohno, T.; Ichikawa, H.; Totoki, Y.; Yasuda, K.; Hiramoto, M.; Nammo, T.; Sakamoto, H.; Tsuta, K.; Furuta, K.; Shimada, Y.; Iwakawa, R.; Ogiwara, H.; Oike, T.; Enari, M.; Schetter, A. J.; Okayama, H.; Haugen, A.; Skaug, V.; Chiku, S.; Yamanaka, I.; Arai, Y.; Watanabe, S.; Sekine, I.; Ogawa, S.; Harris, C. C.; Tsuda, H.; Yoshida, T.; Yokota, J.; Shibata, T., KIF5B-RET fusions in lung adenocarcinoma. *Nat Med* **2012,** *18* (3), 375-7.

[221] Wang, R.; Hu, H.; Pan, Y.; Li, Y.; Ye, T.; Li, C.; Luo, X.; Wang, L.; Li, H.; Zhang, Y.; Li, F.; Lu, Y.; Lu, Q.; Xu, J.; Garfield, D.; Shen, L.; Ji, H.; Pao, W.; Sun, Y.; Chen, H., RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. *J Clin Oncol* **2012,** *30* (35), 4352-9.

[222] Tsuta, K.; Kohno, T.; Yoshida, A.; Shimada, Y.; Asamura, H.; Furuta, K.; Kushima, R., RET-rearranged non-small-cell lung carcinoma: a clinicopathological and molecular analysis. *Br J Cancer* **2014,** *110* (6), 1571-8.

[223] Tamura, T.; Minami, H.; Yamada, Y.; Yamamoto, N.; Shimoyama, T.; Murakami, H.; Horiike, A.; Fujisaka, Y.; Shinkai, T.; Tahara, M.; Kawada, K.; Ebi, H.; Sasaki, Y.; Jiang, H.; Saijo, N., A phase I dose-escalation study of ZD6474 in Japanese patients with solid, malignant tumors. *J Thorac Oncol* **2006,** *1* (9), 1002-9.

[224] Yoh, K.; Seto, T.; Satouchi, M.; Nishio, M.; Yamamoto, N.; Murakami, H.; Nogami, N.; Matsumoto, S.; Kohno, T.; Tsuta, K.; Tsuchihara, K.; Ishii, G.; Nomura, S.; Sato, A.; Ohtsu, A.; Ohe, Y.; Goto, K., Vandetanib in patients with previously treated RET-rearranged advanced non-small-cell lung cancer (LURET): an open-label, multicentre phase 2 trial. *Lancet Respir Med* **2017,** *5* (1), 42-50.

[225] Lee, S. H.; Lee, J. K.; Ahn, M. J.; Kim, D. W.; Sun, J. M.; Keam, B.; Kim, T. M.; Heo, D. S.; Ahn, J. S.; Choi, Y. L.; Min, H. S.; Jeon, Y. K.; Park, K., Vandetanib in pretreated patients with advanced non-small cell lung cancer-harboring RET rearrangement: a phase II clinical trial. *Ann Oncol* **2017,** *28* (2), 292-297.

[226] Subbiah, V.; Velcheti, V.; Tuch, B. B.; Ebata, K.; Busaidy, N. L.; Cabanillas, M. E.; Wirth, L. J.; Stock, S.; Smith, S.; Lauriault, V.; Corsi-Travali, S.; Henry, D.; Burkard, M.; Hamor, R.; Bouhana, K.; Winski, S.; Wallace, R. D.; Hartley, D.; Rhodes, S.; Reddy, M.; Brandhuber, B. J.; Andrews, S.; Rothenberg, S. M.; Drilon, A., Selective RET Kinase Inhibition for Patients with RET-Altered Cancers. *Ann Oncol* **2018**.

[227] Drilon, A. E.; Subbiah, V.; Oxnard, G. R.; Bauer, T. M.; Velcheti, V.; Lakhani, N. J.; Besse, B.; Park, K.; Patel, J. D.; Cabanillas, M. E.; Johnson, M. L.; Reckamp, K. L.; Boni, V.; Loong, H. H. F.; Schlumberger, M.; Solomon, B.; Cruickshank, S.; Rothenberg, S. M.; Shah, M. H.; Wirth, L. J., A phase 1 study of LOXO-292, a potent and highly selective RET inhibitor, in patients with RET-altered cancers. **2018,** *36* (15_suppl), 102-102.

[228] Folkman, J., Tumor angiogenesis: therapeutic implications. *N Engl J Med* **1971,** *285* (21), 1182-6.

[229] Folkman, J., What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* **1990,** *82* (1), 4-6.

[230] Fidler, I. J.; Ellis, L. M., The implications of angiogenesis for the biology and therapy of cancer metastasis. *Cell* **1994,** *79* (2), 185-8.

[231] Wang, J.; Chen, J.; Guo, Y.; Wang, B.; Chu, H., Strategies targeting angiogenesis in advanced non-small cell lung cancer. *Oncotarget* **2017,** *8* (32), 53854-53872.

[232] Kurzrock, R.; Stewart, D. J., Exploring the Benefit/Risk Associated with Antiangiogenic Agents for the Treatment of Non-Small Cell Lung Cancer Patients. *Clin Cancer Res* **2017,** *23* (5), 1137-1148.

[233] Kim, K. J.; Li, B.; Winer, J.; Armanini, M.; Gillett, N.; Phillips, H. S.; Ferrara, N., Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature* **1993,** *362* (6423), 841-4.

[234] Gerber, H. P.; Kowalski, J.; Sherman, D.; Eberhard, D. A.; Ferrara, N., Complete inhibition of rhabdomyosarcoma xenograft growth and neovascularization requires blockade of both tumor and host vascular endothelial growth factor. *Cancer Res* **2000,** *60* (22), 6253-8.

[235] Prewett, M.; Huber, J.; Li, Y.; Santiago, A.; O'Connor, W.; King, K.; Overholser, J.; Hooper, A.; Pytowski, B.; Witte, L.; Bohlen, P.; Hicklin, D. J., Antivascular endothelial growth factor receptor (fetal liver kinase 1) monoclonal antibody inhibits tumor angiogenesis and growth of several mouse and human tumors. *Cancer Res* **1999,** *59* (20), 5209-18.

[236] Bruns, C. J.; Shrader, M.; Harbison, M. T.; Portera, C.; Solorzano, C. C.; Jauch, K. W.; Hicklin, D. J.; Radinsky, R.; Ellis, L. M., Effect of the vascular endothelial growth factor receptor-2 antibody DC101 plus gemcitabine on growth, metastasis and angiogenesis of human pancreatic cancer growing orthotopically in nude mice. *Int J Cancer* **2002,** *102* (2), 101-8.

[237] Zhu, Z.; Lu, D.; Kotanides, H.; Santiago, A.; Jimenez, X.; Simcox, T.; Hicklin, D. J.; Bohlen, P.; Witte, L., Inhibition of vascular endothelial growth factor induced mitogenesis of human endothelial cells by a chimeric anti-kinase insert domain-containing receptor antibody. *Cancer Lett* **1999,** *136* (2), 203-13.

[238] Shaheen, R. M.; Ahmad, S. A.; Liu, W.; Reinmuth, N.; Jung, Y. D.; Tseng, W. W.; Drazan, K. E.; Bucana, C. D.; Hicklin, D. J.; Ellis, L. M., Inhibited growth of colon cancer carcinomatosis by antibodies to vascular endothelial and epidermal growth factor receptors. *Br J Cancer* **2001,** *85* (4), 584-9.

[239] Shaheen, R. M.; Tseng, W. W.; Vellagas, R.; Liu, W.; Ahmad, S. A.; Jung, Y. D.; Reinmuth, N.; Drazan, K. E.; Bucana, C. D.; Hicklin, D. J.; Ellis, L. M., Effects of an antibody to vascular endothelial growth factor receptor-2 on survival, tumor vascularity, and apoptosis in a murine model of colon carcinomatosis. *Int J Oncol* **2001,** *18* (2), 221-6.

[240] Ryu, J. S.; Ryu, H. J.; Lee, S. N.; Memon, A.; Lee, S. K.; Nam, H. S.; Kim, H. J.; Lee, K. H.; Cho, J. H.; Hwang, S. S., Prognostic impact of minimal pleural effusion in non-small-cell lung cancer. *J Clin Oncol* **2014,** *32* (9), 960-7.

[241] Yano, S.; Shinohara, H.; Herbst, R. S.; Kuniyasu, H.; Bucana, C. D.; Ellis, L. M.; Fidler, I. J., Production of experimental malignant pleural effusions is dependent on invasion of the pleura and expression of vascular endothelial growth factor/vascular permeability factor by human lung cancer cells. *Am J Pathol* **2000,** *157* (6), 1893-903.

[242] Kitamura, K.; Kubota, K.; Ando, M.; Takahashi, S.; Nishijima, N.; Sugano, T.; Toyokawa, M.; Miwa, K.; Kosaihira, S.; Noro, R.; Minegishi, Y.; Seike, M.; Yoshimura, A.; Gemma, A., Bevacizumab plus chemotherapy for advanced non-squamous non-small-cell lung cancer with malignant pleural effusion. *Cancer Chemother Pharmacol* **2013,** *71* (2), 457-61.

[243] Yano, S.; Herbst, R. S.; Shinohara, H.; Knighton, B.; Bucana, C. D.; Killion, J. J.; Wood, J.; Fidler, I. J., Treatment for malignant pleural effusion of human lung adenocarcinoma by inhibition of vascular endothelial growth factor receptor tyrosine kinase phosphorylation. *Clin Cancer Res* **2000,** *6* (3), 957-65.

[244] Usui, K.; Sugawara, S.; Nishitsuji, M.; Fujita, Y.; Inoue, A.; Mouri, A.; Watanabe, H.; Sakai, H.; Kinoshita, I.; Ohhara, Y.; Maemondo, M.; Kagamu, H.; Hagiwara, K.; Kobayashi, K.; Group, N. E. J. S., A phase II study of bevacizumab with carboplatin-pemetrexed in non-squamous non-small cell lung carcinoma patients with malignant pleural effusions: North East Japan Study Group Trial NEJ013A. *Lung Cancer* **2016,** *99*, 131-6.

[245] Lima, A. B.; Macedo, L. T.; Sasse, A. D., Addition of bevacizumab to chemotherapy in advanced non-small cell lung cancer: a systematic review and meta-analysis. *PLoS One* **2011,** *6* (8), e22681.

[246] Soria, J. C.; Mauguen, A.; Reck, M.; Sandler, A. B.; Saijo, N.; Johnson, D. H.; Burcoveanu, D.; Fukuoka, M.; Besse, B.; Pignon, J. P.; group, m.-a. o. b. i. a. N. c., Systematic review and meta-analysis of randomised, phase II/III trials adding bevacizumab to platinum-based chemotherapy as first-line treatment in patients with advanced non-small-cell lung cancer. *Ann Oncol* **2013,** *24* (1), 20-30.

[247] Sandler, A. B.; Schiller, J. H.; Gray, R.; Dimery, I.; Brahmer, J.; Samant, M.; Wang, L. I.; Johnson, D. H., Retrospective evaluation of the clinical and radiographic risk factors associated with severe pulmonary hemorrhage in first-line advanced, unresectable non-small-cell lung cancer treated with Carboplatin and Paclitaxel plus bevacizumab. *J Clin Oncol* **2009,** *27* (9), 1405-12.

[248] Johnson, D. H.; Fehrenbacher, L.; Novotny, W. F.; Herbst, R. S.; Nemunaitis, J. J.; Jablons, D. M.; Langer, C. J.; DeVore, R. F.; Gaudreault, J.; Damico, L. A.; Holmgren, E.; Kabbinavar, F., Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* **2004,** *22* (11), 2184-91.

[249] Reck, M.; von Pawel, J.; Zatloukal, P.; Ramlau, R.; Gorbounova, V.; Hirsh, V.; Leighl, N.; Mezger, J.; Archer, V.; Moore, N.; Manegold, C.; Group, B. S., Overall survival with cisplatin-gemcitabine and bevacizumab or placebo as first-line therapy for nonsquamous non-small-cell lung cancer: results from a randomised phase III trial (AVAiL). *Ann Oncol* **2010,** *21* (9), 1804-9.

[250] Zhou, C.; Wu, Y. L.; Chen, G.; Liu, X.; Zhu, Y.; Lu, S.; Feng, J.; He, J.; Han, B.; Wang, J.; Jiang, G.; Hu, C.; Zhang, H.; Cheng, G.; Song, X.; Lu, Y.; Pan, H.; Zheng, W.; Yin, A. Y., BEYOND: A Randomized, Double-Blind, Placebo-Controlled, Multicenter, Phase III Study of First-Line Carboplatin/Paclitaxel Plus Bevacizumab or Placebo in Chinese Patients With Advanced or Recurrent Nonsquamous Non-Small-Cell Lung Cancer. *J Clin Oncol* **2015,** *33* (19), 2197-204.

[251] Socinski, M. A.; Langer, C. J.; Huang, J. E.; Kolb, M. M.; Compton, P.; Wang, L.; Akerley, W., Safety of bevacizumab in patients with non-small-cell lung cancer and brain metastases. *J Clin Oncol* **2009,** *27* (31), 5255-61.

[252] Besse, B.; Le Moulec, S.; Mazières, J.; Senellart, H.; Barlesi, F.; Chouaid, C.; Dansin, E.; Bérard, H.; Falchero, L.; Gervais, R.; Robinet, G.; Ruppert, A. M.; Schott, R.; Léna, H.; Clément-Duchêne, C.; Quantin, X.; Souquet, P. J.; Trédaniel, J.; Moro-Sibilot, D.; Pérol, M.; Madroszyk, A. C.; Soria, J. C., Bevacizumab in Patients with Nonsquamous Non-Small Cell Lung Cancer and Asymptomatic, Untreated Brain Metastases (BRAIN): A Nonrandomized, Phase II Study. *Clin Cancer Res* **2015,** *21* (8), 1896-903.

[253] Barlesi, F.; Gervais, R.; Lena, H.; Hureaux, J.; Berard, H.; Paillotin, D.; Bota, S.; Monnet, I.; Chajara, A.; Robinet, G., Pemetrexed and cisplatin as first-line chemotherapy for advanced non-small-cell lung cancer (NSCLC) with asymptomatic inoperable brain metastases: a multicenter phase II trial (GFPC 07-01). *Ann Oncol* **2011,** *22* (11), 2466-70.

[254] Patel, J. D.; Socinski, M. A.; Garon, E. B.; Reynolds, C. H.; Spigel, D. R.; Olsen, M. R.; Hermann, R. C.; Jotte, R. M.; Beck, T.; Richards, D. A.; Guba, S. C.; Liu, J.; Frimodt-Moller, B.; John, W. J.; Obasaju, C. K.; Pennella, E. J.; Bonomi, P.; Govindan, R., PointBreak: a randomized phase III study of pemetrexed plus carboplatin and bevacizumab followed by maintenance pemetrexed and bevacizumab versus paclitaxel plus carboplatin and bevacizumab followed by maintenance bevacizumab in patients with stage IIIB or IV nonsquamous non-small-cell lung cancer. *J Clin Oncol* **2013,** *31* (34), 4349-57.

[255] Zinner, R. G.; Obasaju, C. K.; Spigel, D. R.; Weaver, R. W.; Beck, J. T.; Waterhouse, D. M.; Modiano, M. R.; Hrinczenko, B.; Nikolinakos, P. G.; Liu, J.; Koustenis, A. G.; Winfree, K. B.; Melemed, S. A.; Guba, S. C.; Ortuzar, W. I.; Desaiah, D.; Treat, J. A.; Govindan, R.; Ross, H. J., PRONOUNCE: randomized, open-label, phase III study of first-line pemetrexed + carboplatin followed by maintenance pemetrexed versus paclitaxel + carboplatin + bevacizumab followed by maintenance bevacizumab in patients ith advanced nonsquamous non-small-cell lung cancer. *J Thorac Oncol* **2015,** *10* (1), 134-42.

[256] Barlesi, F.; Scherpereel, A.; Rittmeyer, A.; Pazzola, A.; Ferrer Tur, N.; Kim, J. H.; Ahn, M. J.; Aerts, J. G.; Gorbunova, V.; Vikström, A.; Wong, E. K.; Perez-Moreno, P.; Mitchell, L.; Groen, H. J., Randomized phase III trial of maintenance bevacizumab with or without pemetrexed after first-line induction with bevacizumab, cisplatin, and pemetrexed in advanced nonsquamous non-small-cell lung cancer: AVAPERL (MO22089). *J Clin Oncol* **2013,** *31* (24), 3004-11.

[257] Takeda, M.; Yamanaka, T.; Seto, T.; Hayashi, H.; Azuma, K.; Okada, M.; Sugawara, S.; Daga, H.; Hirashima, T.; Yonesaka, K.; Urata, Y.; Murakami, H.; Saito, H.; Kubo, A.; Sawa, T.; Miyahara, E.; Nogami, N.; Nakagawa, K.; Nakanishi, Y.; Okamoto,

I., Bevacizumab beyond disease progression after first-line treatment with bevacizumab plus chemotherapy in advanced nonsquamous non-small cell lung cancer (West Japan Oncology Group 5910L): An open-label, randomized, phase 2 trial. *Cancer* **2016,** *122* (7), 1050-9.

[258] Bennouna, J.; Castro, J. D.; Dingemans, A.-M. C.; Griesinger, F.; Grossi, F.; Langer, C. J.; Ohe, Y.; Syrigos, K. N.; Thatcher, N.; Das-Gupta, A.; Donica, M.; Smoljanovic, V.; Gridelli, C., Efficacy and safety results from AvaALL: An open-label, randomized phase III trial of standard of care (SOC) with or without continuous bevacizumab (Bev) treatment beyond progression (PD) in patients (pts) with advanced non-small cell lung cancer (NSCLC) progressing after first-line Bev and chemotherapy (chemo). **2017,** *35* (15_suppl), 9004-9004.

[259] Karkkainen, M. J.; Haiko, P.; Sainio, K.; Partanen, J.; Taipale, J.; Petrova, T. V.; Jeltsch, M.; Jackson, D. G.; Talikka, M.; Rauvala, H.; Betsholtz, C.; Alitalo, K., Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. *Nat Immunol* **2004,** *5* (1), 74-80.

[260] Jeltsch, M.; Kaipainen, A.; Joukov, V.; Meng, X.; Lakso, M.; Rauvala, H.; Swartz, M.; Fukumura, D.; Jain, R. K.; Alitalo, K., Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. *Science* **1997,** *276* (5317), 1423-5.

[261] Veikkola, T.; Jussila, L.; Makinen, T.; Karpanen, T.; Jeltsch, M.; Petrova, T. V.; Kubo, H.; Thurston, G.; McDonald, D. M.; Achen, M. G.; Stacker, S. A.; Alitalo, K., Signalling via vascular endothelial growth factor receptor-3 is sufficient for lymphangiogenesis in transgenic mice. *EMBO J* **2001,** *20* (6), 1223-31.

[262] Hu, X.; Luo, J., Heterogeneity of tumor lymphangiogenesis: progress and prospects. *Cancer Sci* **2018**.

[263] Reck, M.; Paz-Ares, L.; Bidoli, P.; Cappuzzo, F.; Dakhil, S.; Moro-Sibilot, D.; Borghaei, H.; Johnson, M.; Jotte, R.; Pennell, N. A.; Shepherd, F. A.; Tsao, A.; Thomas, M.; Carter, G. C.; Chan-Diehl, F.; Alexandris, E.; Lee, P.; Zimmermann, A.; Sashegyi, A.; Pérol, M., Outcomes in patients with aggressive or refractory disease from REVEL: A randomized phase III study of docetaxel with ramucirumab or placebo for second-line treatment of stage IV non-small-cell lung cancer. *Lung Cancer* **2017,** *112*, 181-187.

[264] Schreiber, R. D.; Old, L. J.; Smyth, M. J., Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* **2011,** *331* (6024), 1565-70.

[265] Chen, D. S.; Mellman, I., Oncology meets immunology: the cancer-immunity cycle. *Immunity* **2013,** *39* (1), 1-10.

[266] Pardoll, D. M., The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* **2012,** *12* (4), 252-64.

[267] Garon, E. B.; Rizvi, N. A.; Hui, R.; Leighl, N.; Balmanoukian, A. S.; Eder, J. P.; Patnaik, A.; Aggarwal, C.; Gubens, M.; Horn, L.; Carcereny, E.; Ahn, M. J.; Felip, E.; Lee, J. S.; Hellmann, M. D.; Hamid, O.; Goldman, J. W.; Soria, J. C.; Dolled-Filhart, M.; Rutledge, R. Z.; Zhang, J.; Lunceford, J. K.; Rangwala, R.; Lubiniecki, G. M.; Roach, C.; Emancipator, K.; Gandhi, L.; Investigators, K.-. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* **2015,** *372* (21), 2018-28.

[268] McLaughlin, J.; Han, G.; Schalper, K. A.; Carvajal-Hausdorf, D.; Pelekanou, V.; Rehman, J.; Velcheti, V.; Herbst, R.; LoRusso, P.; Rimm, D. L., Quantitative Assessment of the Heterogeneity of PD-L1 Expression in Non-Small-Cell Lung Cancer. *JAMA Oncol* **2016,** *2* (1), 46-54.

[269] Sheng, J.; Fang, W.; Yu, J.; Chen, N.; Zhan, J.; Ma, Y.; Yang, Y.; Huang, Y.; Yanhuang; Zhao, H.; Zhang, L., Expression of programmed death ligand-1 on tumor cells varies pre and post chemotherapy in non-small cell lung cancer. *Sci Rep* **2016,** *6*, 20090.

[270] Lin, K.; Cheng, J.; Yang, T.; Li, Y.; Zhu, B., EGFR-TKI down-regulates PD-L1 in EGFR mutant NSCLC through inhibiting NF-κB. *Biochem Biophys Res Commun* **2015,** *463* (1-2), 95-101.

[271] Grigg, C.; Rizvi, N. A., PD-L1 biomarker testing for non-small cell lung cancer: truth or fiction? *J Immunother Cancer* **2016,** *4*, 48.

[272] Sholl, L. M.; Aisner, D. L.; Allen, T. C.; Beasley, M. B.; Borczuk, A. C.; Cagle, P. T.; Capelozzi, V.; Dacic, S.; Hariri, L.; Kerr, K. M.; Lantuejoul, S.; Mino-Kenudson, M.; Raparia, K.; Rekhtman, N.; Roy-Chowdhuri, S.; Thunnissen, E.; Tsao, M. S.; Yatabe, Y.; Society, M. o. P. P., Programmed Death Ligand-1 Immunohistochemistry--A New Challenge for Pathologists: A Perspective From Members of the Pulmonary Pathology Society. *Arch Pathol Lab Med* **2016,** *140* (4), 341-4.

[273] Hansen, A. R.; Siu, L. L., PD-L1 Testing in Cancer: Challenges in Companion Diagnostic Development. *JAMA Oncol* **2016,** *2* (1), 15-6.

[274] Hirsch, F. R.; McElhinny, A.; Stanforth, D.; Ranger-Moore, J.; Jansson, M.; Kulangara, K.; Richardson, W.; Towne, P.; Hanks, D.; Vennapusa, B.; Mistry, A.; Kalamegham, R.; Averbuch, S.; Novotny, J.; Rubin, E.; Emancipator, K.; McCaffery, I.; Williams, J. A.; Walker, J.; Longshore, J.; Tsao, M. S.; Kerr, K. M., PD-L1 Immunohistochemistry Assays for Lung Cancer: Results from Phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project. *J Thorac Oncol* **2017,** *12* (2), 208-222.

[275] Ilie, M.; Long-Mira, E.; Bence, C.; Butori, C.; Lassalle, S.; Bouhlel, L.; Fazzalari, L.; Zahaf, K.; Lalvée, S.; Washetine, K.; Mouroux, J.; Vénissac, N.; Poudenx, M.; Otto, J.; Sabourin, J. C.; Marquette, C. H.; Hofman, V.; Hofman, P., Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of NSCLC patients reveal major discordances: a potential issue for anti-PD-L1 therapeutic strategies. *Ann Oncol* **2016,** *27* (1), 147-53.

[276] Kim, M. Y.; Koh, J.; Kim, S.; Go, H.; Jeon, Y. K.; Chung, D. H., Clinicopathological analysis of PD-L1 and PD-L2 expression in pulmonary squamous cell carcinoma: Comparison with tumor-infiltrating T cells

and the status of oncogenic drivers. *Lung Cancer* **2015,** *88* (1), 24-33.

[277] Hegde, P. S.; Karanikas, V.; Evers, S., The Where, the When, and the How of Immune Monitoring for Cancer Immunotherapies in the Era of Checkpoint Inhibition. *Clin Cancer Res* **2016,** *22* (8), 1865-74.

[278] Rizvi, N. A.; Hellmann, M. D.; Snyder, A.; Kvistborg, P.; Makarov, V.; Havel, J. J.; Lee, W.; Yuan, J.; Wong, P.; Ho, T. S.; Miller, M. L.; Rekhtman, N.; Moreira, A. L.; Ibrahim, F.; Bruggeman, C.; Gasmi, B.; Zappasodi, R.; Maeda, Y.; Sander, C.; Garon, E. B.; Merghoub, T.; Wolchok, J. D.; Schumacher, T. N.; Chan, T. A., Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* **2015,** *348* (6230), 124-8.

[279] Vogelstein, B.; Papadopoulos, N.; Velculescu, V. E.; Zhou, S.; Diaz, L. A.; Kinzler, K. W., Cancer genome landscapes. *Science* **2013,** *339* (6127), 1546-58.

[280] Alexandrov, L. B.; Nik-Zainal, S.; Wedge, D. C.; Aparicio, S. A.; Behjati, S.; Biankin, A. V.; Bignell, G. R.; Bolli, N.; Borg, A.; Børresen-Dale, A. L.; Boyault, S.; Burkhardt, B.; Butler, A. P.; Caldas, C.; Davies, H. R.; Desmedt, C.; Eils, R.; Eyfjörd, J. E.; Foekens, J. A.; Greaves, M.; Hosoda, F.; Hutter, B.; Ilicic, T.; Imbeaud, S.; Imielinski, M.; Imielinsk, M.; Jäger, N.; Jones, D. T.; Jones, D.; Knappskog, S.; Kool, M.; Lakhani, S. R.; López-Otín, C.; Martin, S.; Munshi, N. C.; Nakamura, H.; Northcott, P. A.; Pajic, M.; Papaemmanuil, E.; Paradiso, A.; Pearson, J. V.; Puente, X. S.; Raine, K.; Ramakrishna, M.; Richardson, A. L.; Richter, J.; Rosenstiel, P.; Schlesner, M.; Schumacher, T. N.; Span, P. N.; Teague, J. W.; Totoki, Y.; Tutt, A. N.; Valdés-Mas, R.; van Buuren, M. M.; van 't Veer, L.; Vincent-Salomon, A.; Waddell, N.; Yates, L. R.; Zucman-Rossi, J.; Futreal, P. A.; McDermott, U.; Lichter, P.; Meyerson, M.; Grimmond, S. M.; Siebert, R.; Campo, E.; Shibata, T.; Pfister, S. M.; Campbell, P. J.; Stratton, M. R.; Initiative, A. P. C. G.; Consortium, I. B. C.; Consortium, I. M.-S.; PedBrain, I., Signatures of mutational processes in human cancer. *Nature* **2013,** *500* (7463), 415-21.

[281] Rizvi, H.; Sanchez-Vega, F.; La, K.; Chatila, W.; Jonsson, P.; Halpenny, D.; Plodkowski, A.; Long, N.; Sauter, J. L.; Rekhtman, N.; Hollmann, T.; Schalper, K. A.; Gainor, J. F.; Shen, R.; Ni, A.; Arbour, K. C.; Merghoub, T.; Wolchok, J.; Snyder, A.; Chaft, J. E.; Kris, M. G.; Rudin, C. M.; Socci, N. D.; Berger, M. F.; Taylor, B. S.; Zehir, A.; Solit, D. B.; Arcila, M. E.; Ladanyi, M.; Riely, G. J.; Schultz, N.; Hellmann, M. D., Molecular Determinants of Response to Anti-Programmed Cell Death (PD)-1 and Anti-Programmed Death-Ligand 1 (PD-L1) Blockade in Patients With Non-Small-Cell Lung Cancer Profiled With Targeted Next-Generation Sequencing. *J Clin Oncol* **2018,** *36* (7), 633-641.

[282] Hellmann, M. D.; Nathanson, T.; Rizvi, H.; Creelan, B. C.; Sanchez-Vega, F.; Ahuja, A.; Ni, A.; Novik, J. B.; Mangarin, L. M. B.; Abu-Akeel, M.; Liu, C.; Sauter, J. L.; Rekhtman, N.; Chang, E.; Callahan, M. K.; Chaft, J. E.; Voss, M. H.; Tenet, M.; Li, X. M.; Covello, K.; Renninger, A.; Vitazka, P.; Geese, W. J.; Borghaei, H.; Rudin, C. M.; Antonia, S. J.; Swanton, C.; Hammerbacher, J.; Merghoub, T.; McGranahan, N.; Snyder, A.; Wolchok, J. D., Genomic Features of Response to Combination Immunotherapy in Patients with Advanced Non-Small-Cell Lung Cancer. *Cancer Cell* **2018,** *33* (5), 843-852.e4.

[283] Gubin, M. M.; Esaulova, E.; Ward, J. P.; Malkova, O. N.; Runci, D.; Wong, P.; Noguchi, T.; Arthur, C. D.; Meng, W.; Alspach, E.; Medrano, R. F. V.; Fronick, C.; Fehlings, M.; Newell, E. W.; Fulton, R. S.; Sheehan, K. C. F.; Oh, S. T.; Schreiber, R. D.; Artyomov, M. N., High-Dimensional Analysis Delineates Myeloid and Lymphoid Compartment Remodeling during Successful Immune-Checkpoint Cancer Therapy. *Cell* **2018,** *175* (4), 1014-1030.e19.

[284] Haanen, J. B.; Thienen, H.; Blank, C. U., Toxicity patterns with immunomodulating antibodies and their combinations. *Semin Oncol* **2015,** *42* (3), 423-8.

[285] Weber, J. S.; Yang, J. C.; Atkins, M. B.; Disis, M. L., Toxicities of Immunotherapy for the Practitioner. *J Clin Oncol* **2015,** *33* (18), 2092-9.

[286] Good-Jacobson, K. L.; Szumilas, C. G.; Chen, L.; Sharpe, A. H.; Tomayko, M. M.; Shlomchik, M. J., PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells. *Nat Immunol* **2010,** *11* (6), 535-42.

[287] Zitvogel, L.; Kroemer, G., Targeting PD-1/PD-L1 interactions for cancer immunotherapy. *Oncoimmunology* **2012,** *1* (8), 1223-1225.

[288] Iwama, S.; De Remigis, A.; Callahan, M. K.; Slovin, S. F.; Wolchok, J. D.; Caturegli, P., Pituitary expression of CTLA-4 mediates hypophysitis secondary to administration of CTLA-4 blocking antibody. *Sci Transl Med* **2014,** *6* (230), 230ra45.

[289] Postow, M. A.; Sidlow, R.; Hellmann, M. D., Immune-Related Adverse Events Associated with Immune Checkpoint Blockade. *N Engl J Med* **2018,** *378* (2), 158-168.

[290] Nishino, M.; Giobbie-Hurder, A.; Hatabu, H.; Ramaiya, N. H.; Hodi, F. S., Incidence of Programmed Cell Death 1 Inhibitor-Related Pneumonitis in Patients With Advanced Cancer: A Systematic Review and Meta-analysis. *JAMA Oncol* **2016,** *2* (12), 1607-1616.

[291] Nishino, M.; Ramaiya, N. H.; Awad, M. M.; Sholl, L. M.; Maattala, J. A.; Taibi, M.; Hatabu, H.; Ott, P. A.; Armand, P. F.; Hodi, F. S., PD-1 Inhibitor-Related Pneumonitis in Advanced Cancer Patients: Radiographic Patterns and Clinical Course. *Clin Cancer Res* **2016,** *22* (24), 6051-6060.

[292] Weber, J. S.; Kähler, K. C.; Hauschild, A., Management of immune-related adverse events and kinetics of response with ipilimumab. *J Clin Oncol* **2012,** *30* (21), 2691-7.

[293] Fecher, L. A.; Agarwala, S. S.; Hodi, F. S.; Weber, J. S., Ipilimumab and its toxicities: a multidisciplinary approach. *Oncologist* **2013,** *18* (6), 733-43.

[294] Wellenstein, M. D.; de Visser, K. E., Cancer-Cell-Intrinsic Mechanisms Shaping the Tumor Immune Landscape. *Immunity* **2018,** *48* (3), 399-416.

[295] Quigley, D.; Silwal-Pandit, L.; Dannenfelser, R.; Langerød, A.; Vollan, H. K.; Vaske, C.; Siegel, J. U.; Troyanskaya, O.; Chin, S. F.; Caldas, C.; Balmain, A.; Børresen-Dale, A. L.; Kristensen, V., Lymphocyte Invasion in IC10/Basal-Like Breast Tumors Is Associated with Wild-Type TP53. *Mol Cancer Res* **2015,** *13* (3), 493-501.

[296] Rooney, M. S.; Shukla, S. A.; Wu, C. J.; Getz, G.; Hacohen, N., Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell* **2015,** *160* (1-2), 48-61.

[297] Balli, D.; Rech, A. J.; Stanger, B. Z.; Vonderheide, R. H., Immune Cytolytic Activity Stratifies Molecular Subsets of Human Pancreatic Cancer. *Clin Cancer Res* **2017,** *23* (12), 3129-3138.

[298] Charoentong, P.; Finotello, F.; Angelova, M.; Mayer, C.; Efremova, M.; Rieder, D.; Hackl, H.; Trajanoski, Z., Pan-cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade. *Cell Rep* **2017,** *18* (1), 248-262.

[299] Wang, Q.; Hu, B.; Hu, X.; Kim, H.; Squatrito, M.; Scarpace, L.; deCarvalho, A. C.; Lyu, S.; Li, P.; Li, Y.; Barthel, F.; Cho, H. J.; Lin, Y. H.; Satani, N.; Martinez-Ledesma, E.; Zheng, S.; Chang, E.; Sauvé, C. G.; Olar, A.; Lan, Z. D.; Finocchiaro, G.; Phillips, J. J.; Berger, M. S.; Gabrusiewicz, K. R.; Wang, G.; Eskilsson, E.; Hu, J.; Mikkelsen, T.; DePinho, R. A.; Muller, F.; Heimberger, A. B.; Sulman, E. P.; Nam, D. H.; Verhaak, R. G. W., Tumor Evolution of Glioma-Intrinsic Gene Expression Subtypes Associates with Immunological Changes in the Microenvironment. *Cancer Cell* **2017,** *32* (1), 42-56.e6.

[300] Biton, J.; Mansuet-Lupo, A.; Pécuchet, N.; Alifano, M.; Ouakrim, H.; Arrondeau, J.; Boudou-Rouquette, P.; Goldwasser, F.; Leroy, K.; Goc, J.; Wislez, M.; Germain, C.; Laurent-Puig, P.; Dieu-Nosjean, M. C.; Cremer, I.; Herbst, R.; Blons, H. F.; Damotte, D., *Clin Cancer Res* **2018**.

[301] Skoulidis, F.; Goldberg, M. E.; Greenawalt, D. M.; Hellmann, M. D.; Awad, M. M.; Gainor, J. F.; Schrock, A. B.; Hartmaier, R. J.; Trabucco, S. E.; Gay, L.; Ali, S. M.; Elvin, J. A.; Singal, G.; Ross, J. S.; Fabrizio, D.; Szabo, P. M.; Chang, H.; Sasson, A.; Srinivasan, S.; Kirov, S.; Szustakowski, J.; Vitazka, P.; Edwards, R.; Bufill, J. A.; Sharma, N.; Ou, S. I.; Peled, N.; Spigel, D. R.; Rizvi, H.; Aguilar, E. J.; Carter, B. W.; Erasmus, J.; Halpenny, D. F.; Plodkowski, A. J.; Long, N. M.; Nishino, M.; Denning, W. L.; Galan-Cobo, A.; Hamdi, H.; Hirz, T.; Tong, P.; Wang, J.; Rodriguez-Canales, J.; Villalobos, P. A.; Parra, E. R.; Kalhor, N.; Sholl, L. M.; Sauter, J. L.; Jungbluth, A. A.; Mino-Kenudson, M.; Azimi, R.; Elamin, Y. Y.; Zhang, J.; Leonardi, G. C.; Jiang, F.; Wong, K. K.; Lee, J. J.; Papadimitrakopoulou, V. A.; Wistuba, I. I.; Miller, V. A.; Frampton, G. M.; Wolchok, J. D.; Shaw, A. T.; Jänne, P. A.; Stephens, P. J.; Rudin, C. M.; Geese, W. J.; Albacker, L. A.; Heymach, J. V., Mutations and PD-1 Inhibitor Resistance in. *Cancer Discov* **2018,** *8* (7), 822-835.

[302] Akbay, E. A.; Koyama, S.; Liu, Y.; Dries, R.; Bufe, L. E.; Silkes, M.; Alam, M. M.; Magee, D. M.; Jones, R.; Jinushi, M.; Kulkarni, M.; Carretero, J.; Wang, X.; Warner-Hatten, T.; Cavanaugh, J. D.; Osa, A.; Kumanogoh, A.; Freeman, G. J.; Awad, M. M.; Christiani, D. C.; Bueno, R.; Hammerman, P. S.; Dranoff, G.; Wong, K. K., Interleukin-17A Promotes Lung Tumor Progression through Neutrophil Attraction to Tumor Sites and Mediating Resistance to PD-1 Blockade. *J Thorac Oncol* **2017,** *12* (8), 1268-1279.

[303] Ishida, Y.; Agata, Y.; Shibahara, K.; Honjo, T., Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J* **1992,** *11* (11), 3887-95.

[304] Okazaki, T.; Chikuma, S.; Iwai, Y.; Fagarasan, S.; Honjo, T., A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application. *Nat Immunol* **2013,** *14* (12), 1212-8.

[305] Keir, M. E.; Butte, M. J.; Freeman, G. J.; Sharpe, A. H., PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol* **2008,** *26*, 677-704.

[306] Hamanishi, J.; Mandai, M.; Iwasaki, M.; Okazaki, T.; Tanaka, Y.; Yamaguchi, K.; Higuchi, T.; Yagi, H.; Takakura, K.; Minato, N.; Honjo, T.; Fujii, S., Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. *Proc Natl Acad Sci U S A* **2007,** *104* (9), 3360-5.

[307] Okazaki, T.; Wang, J., PD-1/PD-L pathway and autoimmunity. *Autoimmunity* **2005,** *38* (5), 353-7.

[308] Nishimura, H.; Nose, M.; Hiai, H.; Minato, N.; Honjo, T., Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* **1999,** *11* (2), 141-51.

[309] Nishimura, H.; Okazaki, T.; Tanaka, Y.; Nakatani, K.; Hara, M.; Matsumori, A.; Sasayama, S.; Mizoguchi, A.; Hiai, H.; Minato, N.; Honjo, T., Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* **2001,** *291* (5502), 319-22.

[310] Wang, J.; Yoshida, T.; Nakaki, F.; Hiai, H.; Okazaki, T.; Honjo, T., Establishment of NOD-Pdcd1-/- mice as an efficient animal model of type I diabetes. *Proc Natl Acad Sci U S A* **2005,** *102* (33), 11823-8.

[311] Brahmer, J. R.; Drake, C. G.; Wollner, I.; Powderly, J. D.; Picus, J.; Sharfman, W. H.; Stankevich, E.; Pons, A.; Salay, T. M.; McMiller, T. L.; Gilson, M. M.; Wang, C.; Selby, M.; Taube, J. M.; Anders, R.; Chen, L.; Korman, A. J.; Pardoll, D. M.; Lowy, I.; Topalian, S. L., Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol* **2010,** *28* (19), 3167-75.

[312] Topalian, S. L.; Hodi, F. S.; Brahmer, J. R.; Gettinger, S. N.; Smith, D. C.; McDermott, D. F.; Powderly, J. D.; Carvajal, R. D.; Sosman, J. A.; Atkins, M. B.; Leming, P. D.; Spigel, D. R.; Antonia,

S. J.; Horn, L.; Drake, C. G.; Pardoll, D. M.; Chen, L.; Sharfman, W. H.; Anders, R. A.; Taube, J. M.; McMiller, T. L.; Xu, H.; Korman, A. J.; Jure-Kunkel, M.; Agrawal, S.; McDonald, D.; Kollia, G. D.; Gupta, A.; Wigginton, J. M.; Sznol, M., Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* **2012,** *366* (26), 2443-54.

[313] Brahmer, J.; Reckamp, K. L.; Baas, P.; Crinò, L.; Eberhardt, W. E.; Poddubskaya, E.; Antonia, S.; Pluzanski, A.; Vokes, E. E.; Holgado, E.; Waterhouse, D.; Ready, N.; Gainor, J.; Arén Frontera, O.; Havel, L.; Steins, M.; Garassino, M. C.; Aerts, J. G.; Domine, M.; Paz-Ares, L.; Reck, M.; Baudelet, C.; Harbison, C. T.; Lestini, B.; Spigel, D. R., Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. *N Engl J Med* **2015,** *373* (2), 123-35.

[314] Borghaei, H.; Paz-Ares, L.; Horn, L.; Spigel, D. R.; Steins, M.; Ready, N. E.; Chow, L. Q.; Vokes, E. E.; Felip, E.; Holgado, E.; Barlesi, F.; Kohlhäufl, M.; Arrieta, O.; Burgio, M. A.; Fayette, J.; Lena, H.; Poddubskaya, E.; Gerber, D. E.; Gettinger, S. N.; Rudin, C. M.; Rizvi, N.; Crinò, L.; Blumenschein, G. R.; Antonia, S. J.; Dorange, C.; Harbison, C. T.; Graf Finckenstein, F.; Brahmer, J. R., Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* **2015,** *373* (17), 1627-39.

[315] Carbone, D. P.; Reck, M.; Paz-Ares, L.; Creelan, B.; Horn, L.; Steins, M.; Felip, E.; van den Heuvel, M. M.; Ciuleanu, T. E.; Badin, F.; Ready, N.; Hiltermann, T. J. N.; Nair, S.; Juergens, R.; Peters, S.; Minenza, E.; Wrangle, J. M.; Rodriguez-Abreu, D.; Borghaei, H.; Blumenschein, G. R.; Villaruz, L. C.; Havel, L.; Krejci, J.; Corral Jaime, J.; Chang, H.; Geese, W. J.; Bhagavatheeswaran, P.; Chen, A. C.; Socinski, M. A.; Investigators, C., First-Line Nivolumab in Stage IV or Recurrent Non-Small-Cell Lung Cancer. *N Engl J Med* **2017,** *376* (25), 2415-2426.

[316] Herbst, R. S.; Baas, P.; Kim, D. W.; Felip, E.; Pérez-Gracia, J. L.; Han, J. Y.; Molina, J.; Kim, J. H.; Arvis, C. D.; Ahn, M. J.; Majem, M.; Fidler, M. J.; de Castro, G.; Garrido, M.; Lubiniecki, G. M.; Shentu, Y.; Im, E.; Dolled-Filhart, M.; Garon, E. B., Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* **2016,** *387* (10027), 1540-50.

[317] Reck, M.; Rodríguez-Abreu, D.; Robinson, A. G.; Hui, R.; Csőszi, T.; Fülöp, A.; Gottfried, M.; Peled, N.; Tafreshi, A.; Cuffe, S.; O'Brien, M.; Rao, S.; Hotta, K.; Leiby, M. A.; Lubiniecki, G. M.; Shentu, Y.; Rangwala, R.; Brahmer, J. R.; Investigators, K.-. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N Engl J Med* **2016,** *375* (19), 1823-1833.

[318] Gandhi, L.; Rodríguez-Abreu, D.; Gadgeel, S.; Esteban, E.; Felip, E.; De Angelis, F.; Domine, M.; Clingan, P.; Hochmair, M. J.; Powell, S. F.; Cheng, S. Y.; Bischoff, H. G.; Peled, N.; Grossi, F.; Jennens, R. R.; Reck, M.; Hui, R.; Garon, E. B.; Boyer, M.; Rubio-Viqueira, B.; Novello, S.; Kurata, T.; Gray, J. E.; Vida, J.; Wei, Z.; Yang, J.; Raftopoulos, H.; Pietanza, M. C.; Garassino, M. C.; Investigators, K.-. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. *N Engl J Med* **2018,** *378* (22), 2078-2092.

[319] Paz-Ares, L. G.; Luft, A.; Tafreshi, A.; Gumus, M.; Mazieres, J.; Hermes, B.; Senler, F. C.; Fülöp, A.; Rodriguez-Cid, J.; Sugawara, S.; Cheng, Y.; Novello, S.; Halmos, B.; Shentu, Y.; Kowalski, D., Phase 3 study of carboplatin-paclitaxel/nab-paclitaxel (Chemo) with or without pembrolizumab (Pembro) for patients (Pts) with metastatic squamous (Sq) non-small cell lung cancer (NSCLC). *Journal of Clinical Oncology* **2018,** *36* (15_suppl), 105-105.

[320] Keir, M. E.; Liang, S. C.; Guleria, I.; Latchman, Y. E.; Qipo, A.; Albacker, L. A.; Koulmanda, M.; Freeman, G. J.; Sayegh, M. H.; Sharpe, A. H., Tissue expression of PD-L1 mediates peripheral T cell tolerance. *J Exp Med* **2006,** *203* (4), 883-95.

[321] Fehrenbacher, L.; Spira, A.; Ballinger, M.; Kowanetz, M.; Vansteenkiste, J.; Mazieres, J.; Park, K.; Smith, D.; Artal-Cortes, A.; Lewanski, C.; Braiteh, F.; Waterkamp, D.; He, P.; Zou, W.; Chen, D. S.; Yi, J.; Sandler, A.; Rittmeyer, A.; Group, P. S., Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. *Lancet* **2016,** *387* (10030), 1837-46.

[322] Rittmeyer, A.; Barlesi, F.; Waterkamp, D.; Park, K.; Ciardiello, F.; von Pawel, J.; Gadgeel, S. M.; Hida, T.; Kowalski, D. M.; Dols, M. C.; Cortinovis, D. L.; Leach, J.; Polikoff, J.; Barrios, C.; Kabbinavar, F.; Frontera, O. A.; De Marinis, F.; Turna, H.; Lee, J. S.; Ballinger, M.; Kowanetz, M.; He, P.; Chen, D. S.; Sandler, A.; Gandara, D. R.; Group, O. S., Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* **2017,** *389* (10066), 255-265.

[323] Socinski, M. A.; Jotte, R. M.; Cappuzzo, F.; Orlandi, F. J.; Stroyakovskiy, D.; Nogami, N.; Rodriguez-Abreu, D.; Moro-Sibilot, D.; Thomas, C. A.; Barlesi, F.; Finley, G. G.; Kelsch, C.; Lee, A.; Coleman, S.; Shen, Y.; Kowanetz, M.; Lopez-Chavez, A.; Sandler, A.; Reck, M., Overall survival (OS) analysis of IMpower150, a randomized Ph 3 study of atezolizumab (atezo) + chemotherapy (chemo) ± bevacizumab (bev) vs chemo + bev in 1L nonsquamous (NSQ) NSCLC. *Journal of Clinical Oncology* **2018,** *36* (15_suppl), 9002-9002.

[324] Jotte, R. M.; Cappuzzo, F.; Vynnychenko, I.; Stroyakovskiy, D.; Abreu, D. R.; Hussein, M. A.; Soo, R. A.; Conter, H. J.; Kozuki, T.; Silva, C.; Graupner, V.; Sun, S.; Lin, R. S.; Kelsch, C.; Kowanetz, M.; Hoang, T.; Sandler, A.; Socinski, M. A., IMpower131: Primary PFS and safety analysis of a randomized phase III study of atezolizumab + carboplatin + paclitaxel or nab-paclitaxel vs carboplatin + nab-paclitaxel as 1L therapy in advanced squamous NSCLC. *Journal of*

Clinical Oncology **2018,** *36* (18_suppl), LBA9000-LBA9000.

[325] Antonia, S. J.; Villegas, A.; Daniel, D.; Vicente, D.; Murakami, S.; Hui, R.; Yokoi, T.; Chiappori, A.; Lee, K. H.; de Wit, M.; Cho, B. C.; Bourhaba, M.; Quantin, X.; Tokito, T.; Mekhail, T.; Planchard, D.; Kim, Y. C.; Karapetis, C. S.; Hiret, S.; Ostoros, G.; Kubota, K.; Gray, J. E.; Paz-Ares, L.; de Castro Carpeño, J.; Wadsworth, C.; Melillo, G.; Jiang, H.; Huang, Y.; Dennis, P. A.; Özgüroğlu, M.; Investigators, P., Durvalumab after Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. *N Engl J Med* **2017,** *377* (20), 1919-1929.

[326] Gulley, J. L.; Rajan, A.; Spigel, D. R.; Iannotti, N.; Chandler, J.; Wong, D. J. L.; Leach, J.; Edenfield, W. J.; Wang, D.; Grote, H. J.; Heydebreck, A. V.; Chin, K.; Cuillerot, J. M.; Kelly, K., Avelumab for patients with previously treated metastatic or recurrent non-small-cell lung cancer (JAVELIN Solid Tumor): dose-expansion cohort of a multicentre, open-label, phase 1b trial. *Lancet Oncol* **2017,** *18* (5), 599-610.

[327] Rudd, C. E.; Taylor, A.; Schneider, H., CD28 and CTLA-4 coreceptor expression and signal transduction. *Immunol Rev* **2009,** *229* (1), 12-26.

[328] Tivol, E. A.; Borriello, F.; Schweitzer, A. N.; Lynch, W. P.; Bluestone, J. A.; Sharpe, A. H., Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* **1995,** *3* (5), 541-7.

[329] Waterhouse, P.; Penninger, J. M.; Timms, E.; Wakeham, A.; Shahinian, A.; Lee, K. P.; Thompson, C. B.; Griesser, H.; Mak, T. W., Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science* **1995,** *270* (5238), 985-8.

[330] Hurwitz, A. A.; Foster, B. A.; Kwon, E. D.; Truong, T.; Choi, E. M.; Greenberg, N. M.; Burg, M. B.; Allison, J. P., Combination immunotherapy of primary prostate cancer in a transgenic mouse model using CTLA-4 blockade. *Cancer Res* **2000,** *60* (9), 2444-8.

[331] Hodi, F. S.; O'Day, S. J.; McDermott, D. F.; Weber, R. W.; Sosman, J. A.; Haanen, J. B.; Gonzalez, R.; Robert, C.; Schadendorf, D.; Hassel, J. C.; Akerley, W.; van den Eertwegh, A. J.; Lutzky, J.; Lorigan, P.; Vaubel, J. M.; Linette, G. P.; Hogg, D.; Ottensmeier, C. H.; Lebbé, C.; Peschel, C.; Quirt, I.; Clark, J. I.; Wolchok, J. D.; Weber, J. S.; Tian, J.; Yellin, M. J.; Nichol, G. M.; Hoos, A.; Urba, W. J., Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* **2010,** *363* (8), 711-23.

[332] Hodi, F. S.; Lee, S.; McDermott, D. F.; Rao, U. N.; Butterfield, L. H.; Tarhini, A. A.; Leming, P.; Puzanov, I.; Shin, D.; Kirkwood, J. M., Ipilimumab plus sargramostim vs ipilimumab alone for treatment of metastatic melanoma: a randomized clinical trial. *JAMA* **2014,** *312* (17), 1744-53.

[333] Ribas, A.; Hodi, F. S.; Callahan, M.; Konto, C.; Wolchok, J., Hepatotoxicity with combination of vemurafenib and ipilimumab. *N Engl J Med* **2013,** *368* (14), 1365-6.

[334] Maker, A. V.; Phan, G. Q.; Attia, P.; Yang, J. C.; Sherry, R. M.; Topalian, S. L.; Kammula, U. S.; Royal, R. E.; Haworth, L. R.; Levy, C.; Kleiner, D.; Mavroukakis, S. A.; Yellin, M.; Rosenberg, S. A., Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte-associated antigen 4 blockade and interleukin 2: a phase I/II study. *Ann Surg Oncol* **2005,** *12* (12), 1005-16.

[335] Robert, C.; Thomas, L.; Bondarenko, I.; O'Day, S.; Weber, J.; Garbe, C.; Lebbe, C.; Baurain, J. F.; Testori, A.; Grob, J. J.; Davidson, N.; Richards, J.; Maio, M.; Hauschild, A.; Miller, W. H.; Gascon, P.; Lotem, M.; Harmankaya, K.; Ibrahim, R.; Francis, S.; Chen, T. T.; Humphrey, R.; Hoos, A.; Wolchok, J. D., Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med* **2011,** *364* (26), 2517-26.

[336] Hellmann, M. D.; Ciuleanu, T. E.; Pluzanski, A.; Lee, J. S.; Otterson, G. A.; Audigier-Valette, C.; Minenza, E.; Linardou, H.; Burgers, S.; Salman, P.; Borghaei, H.; Ramalingam, S. S.; Brahmer, J.; Reck, M.; O'Byrne, K. J.; Geese, W. J.; Green, G.; Chang, H.; Szustakowski, J.; Bhagavatheeswaran, P.; Healey, D.; Fu, Y.; Nathan, F.; Paz-Ares, L., Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. *N Engl J Med* **2018,** *378* (22), 2093-2104.

[337] Tarhini, A. A.; Kirkwood, J. M., Tremelimumab, a fully human monoclonal IgG2 antibody against CTLA4 for the potential treatment of cancer. *Curr Opin Mol Ther* **2007,** *9* (5), 505-14.

[338] Antonia, S.; Goldberg, S. B.; Balmanoukian, A.; Chaft, J. E.; Sanborn, R. E.; Gupta, A.; Narwal, R.; Steele, K.; Gu, Y.; Karakunnel, J. J.; Rizvi, N. A., Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study. *Lancet Oncol* **2016,** *17* (3), 299-308.

[339] Lih, C. J.; Harrington, R. D.; Sims, D. J.; Harper, K. N.; Bouk, C. H.; Datta, V.; Yau, J.; Singh, R. R.; Routbort, M. J.; Luthra, R.; Patel, K. P.; Mantha, G. S.; Krishnamurthy, S.; Ronski, K.; Walther, Z.; Finberg, K. E.; Canosa, S.; Robinson, H.; Raymond, A.; Le, L. P.; McShane, L. M.; Polley, E. C.; Conley, B. A.; Doroshow, J. H.; Iafrate, A. J.; Sklar, J. L.; Hamilton, S. R.; Williams, P. M., Analytical Validation of the Next-Generation Sequencing Assay for a Nationwide Signal-Finding Clinical Trial: Molecular Analysis for Therapy Choice Clinical Trial. *J Mol Diagn* **2017,** *19* (2), 313-327.

[340] Pennell, N. A.; Mutebi, A.; Zhou, Z.-Y.; Ricculli, M. L.; Tang, W.; Wang, H.; Guerin, A.; Arnhart, T.; Culver, K. W.; Otterson, G. A., Economic impact of next generation sequencing vs sequential single-gene testing modalities to detect genomic alterations in metastatic non-small cell lung cancer using a decision analytic model. *Journal of Clinical Oncology* **2018,** *36* (15_suppl), 9031-9031.

[341] Gandara, D. R.; Paul, S. M.; Kowanetz, M.; Schleifman, E.; Zou, W.; Li, Y.; Rittmeyer, A.; Fehrenbacher, L.; Otto, G.; Malboeuf, C.; Lieber, D. S.; Lipson, D.; Silterra, J.; Amler, L.; Riehl, T.; Cummings, C. A.; Hegde, P. S.; Sandler, A.; Ballinger, M.; Fabrizio, D.; Mok, T.; Shames, D. S., Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. *Nat Med* **2018**.

[342] Crystal, A. S.; Shaw, A. T.; Sequist, L. V.; Friboulet, L.; Niederst, M. J.; Lockerman, E. L.; Frias, R. L.; Gainor, J. F.; Amzallag, A.; Greninger, P.; Lee, D.; Kalsy, A.; Gomez-Caraballo, M.; Elamine, L.; Howe, E.; Hur, W.; Lifshits, E.; Robinson, H. E.; Katayama, R.; Faber, A. C.; Awad, M. M.; Ramaswamy, S.; Mino-Kenudson, M.; Iafrate, A. J.; Benes, C. H.; Engelman, J. A., Patient-derived models of acquired resistance can identify effective drug combinations for cancer. *Science* **2014,** *346* (6216), 1480-6.

[343] Ohashi, Y.; Okamura, M.; Katayama, R.; Fang, S.; Tsutsui, S.; Akatsuka, A.; Shan, M.; Choi, H. W.; Fujita, N.; Yoshimatsu, K.; Shiina, I.; Yamori, T.; Dan, S., Targeting the Golgi apparatus to overcome acquired resistance of non-small cell lung cancer cells to EGFR tyrosine kinase inhibitors. *Oncotarget* **2018,** *9* (2), 1641-1655.

[344] Tanimoto, A.; Takeuchi, S.; Arai, S.; Fukuda, K.; Yamada, T.; Roca, X.; Ong, S. T.; Yano, S., Histone Deacetylase 3 Inhibition Overcomes. *Clin Cancer Res* **2017,** *23* (12), 3139-3149.

[345] Chen, H.; Wang, Y.; Lin, C.; Lu, C.; Han, R.; Jiao, L.; Li, L.; He, Y., Vorinostat and metformin sensitize EGFR-TKI resistant NSCLC cells via BIM-dependent apoptosis induction. *Oncotarget* **2017,** *8* (55), 93825-93838.

[346] Suda, K.; Sato, K.; Mizuuchi, H.; Kobayashi, Y.; Shimoji, M.; Tomizawa, K.; Takemoto, T.; Iwasaki, T.; Sakaguchi, M.; Mitsudomi, T., Recent evidence, advances, and current practices in surgical treatment of lung cancer. *Respir Investig* **2014,** *52* (6), 322-9.

[347] Postoperative radiotherapy in non-small-cell lung cancer: systematic review and meta-analysis of individual patient data from nine randomised controlled trials. PORT Meta-analysis Trialists Group. *Lancet* **1998,** *352* (9124), 257-63.

[348] Douillard, J. Y.; Rosell, R.; De Lena, M.; Carpagnano, F.; Ramlau, R.; Gonzáles-Larriba, J. L.; Grodzki, T.; Pereira, J. R.; Le Groumellec, A.; Lorusso, V.; Clary, C.; Torres, A. J.; Dahabreh, J.; Souquet, P. J.; Astudillo, J.; Fournel, P.; Artal-Cortes, A.; Jassem, J.; Koubkova, L.; His, P.; Riggi, M.; Hurteloup, P., Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. *Lancet Oncol* **2006,** *7* (9), 719-27.

[349] Pignon, J. P.; Tribodet, H.; Scagliotti, G. V.; Douillard, J. Y.; Shepherd, F. A.; Stephens, R. J.; Dunant, A.; Torri, V.; Rosell, R.; Seymour, L.; Spiro, S. G.; Rolland, E.; Fossati, R.; Aubert, D.; Ding, K.; Waller, D.; Le Chevalier, T.; Group, L. C., Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. *J Clin Oncol* **2008,** *26* (21), 3552-9.

[350] Pollock, R. E.; Roth, J. A., Cancer-induced immunosuppression: implications for therapy? *Semin Surg Oncol* **1989,** *5* (6), 414-9.

[351] Kadosawa, T.; Watabe, A., The effects of surgery-induced immunosuppression and angiogenesis on tumour growth. *Vet J* **2015,** *205* (2), 175-9.

[352] Gobbini, E.; Giaj Levra, M., Is there a room for immune checkpoint inhibitors in early stage non-small cell lung cancer? *J Thorac Dis* **2018,** *10* (Suppl 13), S1427-S1437.

[353] Demaria, S.; Formenti, S. C., Radiation as an immunological adjuvant: current evidence on dose and fractionation. *Front Oncol* **2012,** *2*, 153.

[354] Kaur, P.; Asea, A., Radiation-induced effects and the immune system in cancer. *Front Oncol* **2012,** *2*, 191.

[355] Krysko, D. V.; Garg, A. D.; Kaczmarek, A.; Krysko, O.; Agostinis, P.; Vandenabeele, P., Immunogenic cell death and DAMPs in cancer therapy. *Nat Rev Cancer* **2012,** *12* (12), 860-75.

[356] Green, D. R.; Ferguson, T.; Zitvogel, L.; Kroemer, G., Immunogenic and tolerogenic cell death. *Nat Rev Immunol* **2009,** *9* (5), 353-63.

[357] Demaria, S.; Golden, E. B.; Formenti, S. C., Role of Local Radiation Therapy in Cancer Immunotherapy. *JAMA Oncol* **2015,** *1* (9), 1325-32.

[358] Chandra, R. A.; Wilhite, T. J.; Balboni, T. A.; Alexander, B. M.; Spektor, A.; Ott, P. A.; Ng, A. K.; Hodi, F. S.; Schoenfeld, J. D., A systematic evaluation of abscopal responses following radiotherapy in patients with metastatic melanoma treated with ipilimumab. *Oncoimmunology* **2015,** *4* (11), e1046028.

[359] Demaria, M.; Ohtani, N.; Youssef, S. A.; Rodier, F.; Toussaint, W.; Mitchell, J. R.; Laberge, R. M.; Vijg, J.; Van Steeg, H.; Dollé, M. E.; Hoeijmakers, J. H.; de Bruin, A.; Hara, E.; Campisi, J., An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev Cell* **2014,** *31* (6), 722-33.

[360] Demaria, S.; Ng, B.; Devitt, M. L.; Babb, J. S.; Kawashima, N.; Liebes, L.; Formenti, S. C., Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int J Radiat Oncol Biol Phys* **2004,** *58* (3), 862-70.

[361] MOLE, R. H., Whole body irradiation; radiobiology or medicine? *Br J Radiol* **1953,** *26* (305), 234-41.

[362] Okuma, K.; Yamashita, H.; Niibe, Y.; Hayakawa, K.; Nakagawa, K., Abscopal effect of radiation on lung metastases of hepatocellular carcinoma: a case report. *J Med Case Rep* **2011,** *5*, 111.

[363] Wersäll, P. J.; Blomgren, H.; Pisa, P.; Lax, I.; Kälkner, K. M.; Svedman, C., Regression of non-irradiated metastases after extracranial stereotactic radiotherapy in metastatic renal cell carcinoma. *Acta Oncol* **2006,** *45* (4), 493-7.

[364] Sharabi, A. B.; Lim, M.; DeWeese, T. L.; Drake, C. G., Radiation and checkpoint blockade

immunotherapy: radiosensitisation and potential mechanisms of synergy. *Lancet Oncol* **2015,** *16* (13), e498-509.

[365] Tsakonas, G.; Ekman, S., Oncogene-addicted non-small cell lung cancer and immunotherapy. *J Thorac Dis* **2018,** *10* (Suppl 13), S1547-S1555.

[366] Mazieres, J.; Drilon, A. E.; Mhanna, L.; Milia, J.; Lusque, A.; Cortot, A. B.; Mezquita, L.; Thai, A.; Couraud, S.; Veillon, R.; Mascaux, C.; Schouten, R.; Neal, J. W.; Ng, T. L.; Frueh, M.; Peled, N.; Gounant, V.; Popat, S.; Zhu, V. W.; Gautschi, O., Efficacy of immune-checkpoint inhibitors (ICI) in non-small cell lung cancer (NSCLC) patients harboring activating molecular alterations (ImmunoTarget). **2018,** *36* (15_suppl), 9010-9010.

[367] Boni, A.; Cogdill, A. P.; Dang, P.; Udayakumar, D.; Njauw, C. N.; Sloss, C. M.; Ferrone, C. R.; Flaherty, K. T.; Lawrence, D. P.; Fisher, D. E.; Tsao, H.; Wargo, J. A., Selective BRAFV600E inhibition enhances T-cell recognition of melanoma without affecting lymphocyte function. *Cancer Res* **2010,** *70* (13), 5213-9.

[368] Kono, M.; Dunn, I. S.; Durda, P. J.; Butera, D.; Rose, L. B.; Haggerty, T. J.; Benson, E. M.; Kurnick, J. T., Role of the mitogen-activated protein kinase signaling pathway in the regulation of human melanocytic antigen expression. *Mol Cancer Res* **2006,** *4* (10), 779-92.

[369] Frederick, D. T.; Piris, A.; Cogdill, A. P.; Cooper, Z. A.; Lezcano, C.; Ferrone, C. R.; Mitra, D.; Boni, A.; Newton, L. P.; Liu, C.; Peng, W.; Sullivan, R. J.; Lawrence, D. P.; Hodi, F. S.; Overwijk, W. W.; Lizée, G.; Murphy, G. F.; Hwu, P.; Flaherty, K. T.; Fisher, D. E.; Wargo, J. A., BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. *Clin Cancer Res* **2013,** *19* (5), 1225-31.

[370] Liu, C.; Peng, W.; Xu, C.; Lou, Y.; Zhang, M.; Wargo, J. A.; Chen, J. Q.; Li, H. S.; Watowich, S. S.; Yang, Y.; Tompers Frederick, D.; Cooper, Z. A.; Mbofung, R. M.; Whittington, M.; Flaherty, K. T.; Woodman, S. E.; Davies, M. A.; Radvanyi, L. G.; Overwijk, W. W.; Lizée, G.; Hwu, P., BRAF inhibition increases tumor infiltration by T cells and enhances the antitumor activity of adoptive immunotherapy in mice. *Clin Cancer Res* **2013,** *19* (2), 393-403.

[371] Callahan, M. K.; Masters, G.; Pratilas, C. A.; Ariyan, C.; Katz, J.; Kitano, S.; Russell, V.; Gordon, R. A.; Vyas, S.; Yuan, J.; Gupta, A.; Wigginton, J. M.; Rosen, N.; Merghoub, T.; Jure-Kunkel, M.; Wolchok, J. D., Paradoxical activation of T cells via augmented ERK signaling mediated by a RAF inhibitor. *Cancer Immunol Res* **2014,** *2* (1), 70-9.

[372] Jiang, X.; Zhou, J.; Giobbie-Hurder, A.; Wargo, J.; Hodi, F. S., The activation of MAPK in melanoma cells resistant to BRAF inhibition promotes PD-L1 expression that is reversible by MEK and PI3K inhibition. *Clin Cancer Res* **2013,** *19* (3), 598-609.

[373] Atefi, M.; Avramis, E.; Lassen, A.; Wong, D. J.; Robert, L.; Foulad, D.; Cerniglia, M.; Titz, B.; Chodon, T.; Graeber, T. G.; Comin-Anduix, B.; Ribas, A., Effects of MAPK and PI3K pathways on PD-L1 expression in melanoma. *Clin Cancer Res* **2014,** *20* (13), 3446-57.

[374] Puzanov, I.; Callahan, M. K.; Linette, G. P.; Patel, S. P.; Luke, J. J.; Sosman, J. A.; Wolchok, J. D.; Hamid, O.; Minor, D. R.; Orford, K. W.; Hug, B. A.; Ma, B.; Matthys, G. M.; Hoos, A., Phase 1 study of the BRAF inhibitor dabrafenib (D) with or without the MEK inhibitor trametinib (T) in combination with ipilimumab (Ipi) for V600E/K mutation–positive unresectable or metastatic melanoma (MM). **2014,** *32* (15_suppl), 2511-2511.

[375] Kerbel, R. S.; Kamen, B. A., The anti-angiogenic basis of metronomic chemotherapy. *Nat Rev Cancer* **2004,** *4* (6), 423-36.

[376] Maiti, R., Metronomic chemotherapy. *J Pharmacol Pharmacother* **2014,** *5* (3), 186-92.

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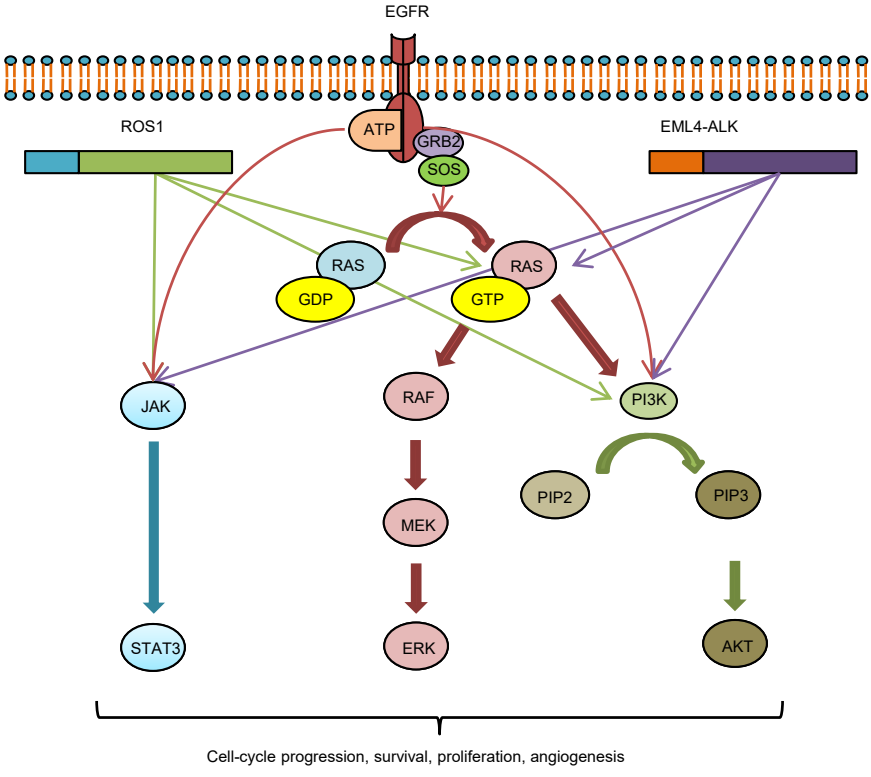
EGFR
ROS1
ATP
GRB2
SOS
EML4-ALK
RAS
GDP
RAS
GTP
JAK
RAF
PI3K
PIP2
PIP3
MEK
STAT3
ERK
AKT
Cell-cycle progression, survival, proliferation, angiogenesis

Table 1. Molecular targeted therapies and their molecular targets

Molecular target therapies	Generation	Drug	Molecular target	Acquired mutations
EGFR-TKI	First	Gefitinib	*EGFR* L858R, Del 19	*EGFR* T790M, *HER2* amplifiaction, *MET* amplification
	First	Erlotinib	*EGFR* L858R, Del 19	*EGFR* T790M, *HER2* amplifiaction, *MET* amplification
	Second	Afatinib	*EGFR* L858R, Del 19, G719X, S768I, L861Q, Wild type-*HER2* , *HER2*	*EGFR* T790M, *HER2* amplifiaction, *MET* amplification
	Third	Osimertinib	*EGFR* L858R, Del 19, T790M, G719A/C/D/X, S768I, L861Q	*EGFR* C797S, Wild type *EGFR* amplification, Loss of T790M and other activated mutation, *MET* amplification, *HER2* amplifiaction, *FGFR1* amplification, *KRAS* mutation, *PIK3CA* mutation
ALK-TKI	First	Crizotinib	*EML4-ALK* , *MET* , *ROS1*	*ALK* L1196M, C1156Y, G1269A, F1174L, I151Tins, L1152R, S1206Y, I1171T, V1180L, D1203N, G1202R, *EGFR* mutation, *KRAS* mutation, *KIT*
	Second	Alectinib	*EML4-ALK* , *ALK* C1156Y, I1171T, F1174C, L1198F, D1203N, E1210K,	*ALK* V1180L, I1171N/S/T, G1202R, *MET* amplification
	Second	Ceritinib	*EML4-ALK* , *ALK* C1156Y, I1171N/S/T, F1174C, L1196M, D1203N, E1210K, G1269A	*ALK* C1156Y, F1174C/V, L1152R, G1202R, G1123S
ROS1-TKI		Crizotinib	*EML4-ALK* , *MET* , *ROS1*	*ROS1* G2032R, D2033N, S1986Y, S1986F
BRAF-TKI		Dabrafenib	*BRAF* V600E	
		Trametinib	*MEK*	
RET-TKI		Vandetanib	*VEGF* , *EGFR* , *RET*	
Anti-VEGF antibody		Bevacizumab	VEGF-A	
Anti-VEGFR2 antibody		Ramucirumab	VEGFR-2	
Anti-PD-1 antibody		Nivolumab	PD-1	
		Pembrolizumab	PD-1	
Anti-PD-L1 antibody		Atezolizumab	PD-L1	
		Durvalumab	PD-L1	
Anti-CTLA-4 antibody		Ipilimumab	CTLA-4	

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; HER2, human epidermal growth factor receptor 2; MET, mesenchymal–epithelial transition factor; FGFR1, fibroblast growth factor receptor 1; KRAS, Kirsten rat sarcoma viral oncogene homolog; PIK3CA, phosphoinositide-3-kinase P110α catalytic subunitALK, anaplastic lymphoma kinase; EML4, echinoderm microtubule-associated protein-like 4; ROS1, c-ros oncogene 1; KIT, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; BRAF, v-Raf murine sarcoma viral oncogene homolog B; MEK, mitogen-activated protein kinase kinase; RET, rearranged during transfection; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated

Table 2. Phase III tirals comparing first-line EGFR-TKI and platinum-doublet for activated *EGFR* mutation positive NSCLC

Trial	Cases	Regimen	RR (%)	PFS (months)	HR	OS (months)	HR
IPASS	261	Gefitinib vs. CBDCA/PTX	71 vs. 47	9.5 vs. 6.3	0.48 (0.36-0.64) *P* <0.0001	22.8 vs. 20.3	1.00 (0.76-1.13)
NEJ002	228	Gefitinib vs. CBDCA/PTX	74 vs. 31	10.8 vs. 5.4	0.30 (0.22-0.41) *P* <0.001	30.5 vs. 23.6	0.89 (0.63-1.24) *P* =0.31
WJTOG3405	172	Gefitinib vs. CDDP/DTX	62 vs. 32	9.6 vs. 6.6	0.56 (0.41-0.77) *P* <0.0001	35.5 vs. 38.8	1.185 (0.767-1.829) *P* =0.443
First-SIGNAL	42	Gefitinib vs. CDDP/GEM	85 vs. 38	8.0 vs. 6.3	0.54 (0.27-1.1)	27.2 vs 25.6	1.04 (0.50-2.2)
EURTAC	174	Erlotinib vs. CDDP or CBDCA/DTX or GEM	61 vs. 18	9.7 vs. 5.2	0.37 (0.25-0.54) *P* <0.0001	22.9 vs. 19.6	0.92 (0.63-1.35)
OPTIMAL	165	Erlotinib vs. CBDCA/GEM	83 vs. 36	13.7 vs. 4.6	0.16 (0.11-0.26) *P* <0.0001	22.8 vs. 27.2	1.19 (0.83-1.71) *P* =0.2663
Lux-Lung 3	345	Afatinib vs. CDDP/PEM	56 vs. 23	11.1 vs. 6.9	0.58 (0.43-0.78) *P* =0.001	28.2 vs. 28.2	0.88 (0.66-1.17) *P* =0.39
Lux-Lung 6	363	Afatinib vs. CDDP/GEM	74 vs. 31	11.0 vs. 5.6	0.28 (0.20-0.39) *P* <0.0001	23.1 vs. 23.5	0.93 (0.72-1.22) *P* =0.61
FLAURA	556	Osimertinib vs. gefitinib or erlotinib	80 vs. 76	18.9 vs. 10.2	0.46 (0.37-0.57) *P* <0.001	Not available	Not available

EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; NSCLC, non-small cell lung cancer; RR, response rate; PFS, progression free survival;
HR, hazard ratio; OS, overall survival; CBDCA, calboplatin; PTX, paclitaxel; CDDP, cisplatin; DTX, docetaxel; GEM, gemcitabine; PEM, pemetrexed.

Table 3. CTCAE any grade and grade 3/4 adverse events induced by tyrosine kinase inhibitor

TKI	Trial	Any grade adverse events (percentage)	Grade 3/4 adverse events (percentage)
Gefitinib	WJTOG3405	Rash (85), incleased ALT (70), diarrhea (54)	Rash (2), incleased ALT (28), diarrhea (1)
Erlotinib	OPTIMAL	Rash (73), incleased ALT (37), diarrhea (25)	Rash (2), incleased ALT (4), diarrhea (1)
Afatinib	Lux-Lung 6	Not available	Rash or acne (15), diarrhea (5), stomatitis or mucositis (5)
Osimertinib	FLAURA	Rash or acne (58), diarrhea (58), dry skin (36)	Rash or acne (1), diarrhea (2), dry skin (<1)
Crizotinib	PROFILE1014	Vision disorder (71%), diarrhea (61), nausea (56)	Vision disorder (1), diarrhea (2), nausea (1)
Alectinib	J-ALEX	Constipation (35), nasopharyngitis (20), dysgeusia (18)	Constipation (1), nasopharyngitis (0), dysgeusia (0)
Ceritinib	ASCEND-4	Diarrhea (85), nausea (69), vomitting (66)	Diarrhea (5), nausea (3), vomitting (5)
Dabrafenib		Pyrexia (35), asthenia (30), hyperkeratosis (30)	Pyrexia (2), asthenia (5), hyperkeratosis (1)
Vandetanib	LURET	Not available	Hypertention (58), rash (16), QT prolongation (11), diarrhea (11)

CTCAE, Common terminology criteria for adverse events.

Table 4. Phase III tirals of ALK-TKI in NSCLC patients with *ALK* fusion gene

Trial	Cases	Line	Regimen	RR (%)	PFS (months)	HR	OS (months)	HR
PROFILE1007	347	2	Crizotinib vs. Plutinum-based chemotherapy	65 vs. 20	7.7 vs. 3.0	0.49 (0.37-0.64) *P* <0.001	21.6 vs. 21.9	1.02 (0.68-1.54) *P* =0.54
PROFILE1014	343	1	Crizotinib vs. Chemotherapy	74 vs. 45	10.9 vs. 7.0	0.45 (0.35-0.60) *P* <0.001	Not reached vs.	0.82 (0.54-1.26) *P* =0.36
J-ALEX	207	1 or 2	Alectinib vs. Crizotinib	85 vs. 70	Not reached vs.	0.34 (0.17-0.71) *P* <0.0001	Not reached vs.	Not available
ALEX	303	1	Alectinib vs. Crizotinib	82.9 vs. 75.5	25.7 vs. 10.4	0.50 (0.36-0.70) *P* <0.001	Not reached vs.	0.76 (0.48-1.20) *P* =0.24
ASCEND-4	376	1	Ceritinib vs. Plutinum-based chemotherapy	72.5 vs. 26.7	16.6 vs. 8.1	0.55 (0.42-0.73) *P* <0.00001	Not reached vs.	0.73 (0.50-1.08) *P* =0.056
ASCEND-5	231	3 or 4	Ceritinib vs. PEM or DTX	45 vs. 8	5.4 vs. 1.6	0.49 (0.36-0.67) *P* <0.0001	18.1 vs. 20.1	1.0 (0.67-1.49) *P* =0.50

ALK, anaplastic lymphoma kinase; NSCLC, non-small cell lung cancer; RR, response rate; PFS, progression free survival; HR, hazard ratio; OS, overall survival; PEM, pemetrexed; DTX, docetaxel.

Table 5. Clinica tirals of ROS1-, BRAF- and RET-TKI in NSCLC patients with *ROS1* , *BRAF* and *RET* mutation, respectively

Molecular target	Drug	Phase	Case	RR (%)	PFS (months)	95% CI (months)
ROS1	Crizotinib	2	127	71.7	15.9	12.9-24.0
BRAF	Dabrafenib	2	84	33	5.5	3.4-7.3
	Dabrafenib and trametinib	2	57	66.7	9.7	6.9-19.6
RET	Vandetanib	2	17	53	4.7	2.8-8.5

ROS1, c-ros oncogene 1; BRAF, v-Raf murine sarcoma viral oncogene homolog B; RET, rearranged during transfection; NSCLC, non-small cell lung cancer; RR, response rate; PFS, progression free survival; CI, confidence interval

Table 6. Phase 3 tirals of anti-VEGF antibody and anti-VEGFR2 antibody in NSCLC patients

Target molecule	Trial	Cases	Regimen	RR (%)	PFS (months)	HR	OS (months)	HR
VEGF	ECOG4599	878	Paclitaxel and carboplatin plus bevacizumab vs. paclitaxel and carboplatin	35 vs. 15	6.2 vs. 4.5	0.66 (0.57-0.77) *P*<0.001	12.3 vs. 10.3	0.79 (0.67-0.92) *P*=0.003
	AVAiL	343	Gemcitabine and cisplatin plus bevacizumab (7.5 mg/kg) vs.	34.1 vs. 20.1	6.7 vs. 6.1	0.75 (0.64-0.87) *P*=0.0003	13.6 vs. 13.1	0.93 (0.78-1.11) *P*=0.420
			Gemcitabine and cisplatin plus bevacizumab (15 mg/kg) vs. Gemcitabine and cisplatin plus placebo	30.4 vs. 20.1	6.5 vs. 6.1	0.85 (-0.73-1.00)	13.4 vs. 13.1	1.03 (0.86-1.23) *P*=0.761
	BEYOND	276	Paclitaxel and carboplatin plus bevacizumab vs. paclitaxel and carboplatin plus placebo	54 vs. 26	12.4 vs. 7.9	0.27 (0.12-0.63) *P*<0.0001	24.3 vs. 17.7	0.68 (0.50-0.93) *P*=0.0154
	PointBreak	939	Pemetrexed and carboplatin plus bevacizumab→ pemetrexed and bevacizumab vs. Paclitaxel and carboplatin plus bevacizumab→ bevacizumab	34.1 vs. 33.0	6.0 vs. 5.6	0.83 (0.71-0.96) *P*=0.012	12.6 vs. 13.4	1.00 (0.86-1.1.6) *P*=0.949
	PRONOUNCE	361	Pemetrexed and carboplatin→pemetrexed vs. Paclitaxel and carboplatin plus bevacizumab→ bevacizumab	23.6 vs. 27.4	4.44 vs. 5.49	1.06 (0.84-1.35) *P*=0.61	10.5 vs. 11.7	1.07 (0.83-1.36) *P*=0.615
	AVAPERL	253	Pemetrexed and cisplatin plus bevacizumab→ pemetrexed and bevacizumab vs. Pemetrexed and cisplatin plus bevacizumab→ bevacizumab	50.0 vs. 55.5	10.2 vs. 6.6	0.50 (0.37-0.69) *P*<0.001	17.1 vs. 13.2	0.87 (0.63-1.21) *P*=0.29
	AvaALL	485	Chemotherapy plus bevacizumab→ chemotherapy plus bevacizumab vs. Chemotherapy plus bevacizumab→ chemotherapy	9.7 vs. 6.7	4.9 vs. 3.8 (PFS2)	0.85 (0.72-1.00) *P*=0.0907	11.9 vs. 10.2	0.84 (0.71-1.00) *P*=0.1016
VEGFR2	REVEL	1253	Docetaxel plus ramucirumab vs. docetaxel plus placebo	23 vs. 14	4.5 vs. 3.0	0.76 (0.68-0.86) *P*<0.0001	10.5 vs. 9.1	0.86 (0.75-0.98) *P*=0.023

VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; NSCLC, non-small cell lung cancer; RR, response rate; PFS, progression free survival; HR, hazard ratio; OS, overall survival; PFS2, progression free survival after second progressive disease

Table 7. Phase III tirals of ICIs in NSCLC patients

Trial	Cases	Line	Histology	Regimen	PD-L1 status	RR (%)	PFS (months)	HR	OS (months)	HR
CheckMate 017	272	2	squamous	Nivolumab vs. Docetaxel	any	20 vs. 9	3.5 vs. 2.8	0.62 (0.37-0.64) *P* <0.001	9.2 vs. 6.0	0.59 (0.44-0.79) *P* <0.001
CheckMate 026	423	1	any	Nivolumab vs. Platinum-based chemotherapy	≥5%	26 vs. 33	4.2 s 5.9	1.15 (0.91-1.45) *P* =0.25	14.4 vs. 13.2	1.02 (0.80-1.31)
CheckMate 057	582	2	non-squamous	Nivolumab vs. Docetaxel	any	19 vs. 12	2.3 vs. 4.2	0.92 (0.77-1.1) *P* =0.39	12.2 vs. 9.4	0.73 (0.59-0.89) *P* =0.002
KEYNOTE-010	1034	2	any	Pembrolizumab 2 mg/kg vs. Docetaxel Pembrolizumab 10 mg/kg vs. Docetaxel	any	30.2 vs. 7.9 29.1 vs. 7.9	3.9 vs. 4.0 4.0 vs. 4.0	0.88 (0.74-1.05), *P* =0.07 0.79 (0.66-0.94), *P* =0.004	10.4 vs. 8.5 12.7 vs. 8.5	0.71 (0.58-0.88), *P* =0.0008 0.61 (0.49-0.75), *P* <0.0001
KEYNOTE-024	305	1	any	Pembrolizumab vs. Platinum-based chemotherapy	≥50%	44.8 vs. 27.8	10.3 vs. 6.0	0.50 (0.37-0.68) *P* <0.001	not reached vs.	0.60 (0.41-0.89) *P* =0.005
KEYNOTE-189	616	1	non-squamous	Pembrolizumab plus Platinum-based chemotherapy	any	47.6 vs. 18.9	8.8 vs. 4.9	0.50 (0.37-0.68) *P* <0.001	not reached vs.	0.49 (0.38-0.64) *P*< 0.001
OAK	1225	2	any	Atezolizumab vs. Docetaxel	any	13.6 vs. 13.4	2.8 vs. 4.0	0.95 (0.82-1.10) *P* =0.4928	13.8 vs. 9.6	0.73 (0.62-0.87) *P*= 0.0003
PACIFIC	231	1	any	Durvalumab after chemoradiotherapy vs. Placebo after chemoradiotherapy	any	28.4 vs. 16	16.8 vs. 5.6	0.52 (0.42-0.65) *P* <0.0001	Not available	Not available

ICI, immune checkpoint inhibitor; NSCLC, non-small cell lung cancer; PD-L1, programmed death-ligand 1; RR, response rate; PFS, progression free survival; HR, hazard ratio; OS, overall