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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 neo-vessels[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 there 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 there 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.

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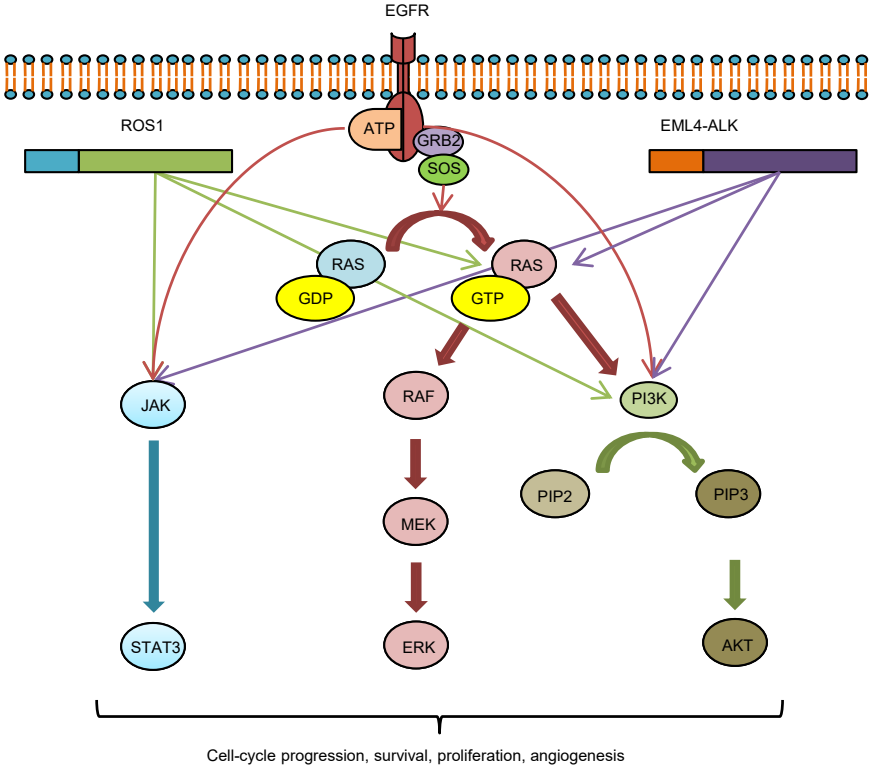
EGFR
ROS1
EML4-ALK
ATP
GRB2
SOS
RAS
GDP
RAS
GTP
JAK
RAF
PI3K
PIP2
PIP3
MEK
STAT3
ERK
AKT
Cell-cycle progression, survival, proliferation, angiogenesis

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