

Progress in Treatment of Non-Small Cell Lung Cancer Harboring HER2 Aberrations

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Abstract: Epidermal growth factor receptor 2 (HER2/ErbB2/neu), a member of ErbB receptor tyrosine kinase family, forms homo- or heterodimers with ErbB1 (HER1/EGFR), ErbB3 (HER3), or ErbB4 (HER4), to activate signal transduction pathways and promote proliferation, differentiation and tumorigenesis. Preliminary clinical trials of monoclonal antibodies, antibody conjugates and small-molecule tyrosine kinase inhibitors targeting HER2 have indicated that HER2 is a potential therapeutic target in non-small cell lung cancer (NSCLC). HER2 aberrations in NSCLC patients mainly include mutation, amplification, and overexpression. While there are significant differences in the outcome of NSCLC with these HER2 changes, no consensus has been reached for the incidence, detection method and targeted treatments for the three types of HER2 aberration. HER2 mutation is generally considered to have more clinical relevance and response to HER2-targeted therapies. In this review, we discuss HER2 alterations in NSCLC, including diagnostic challenges and treatment strategies particular to the HER2 mutation.

Keywords: HER2, non-small cell lung cancer, antibody conjugate, tyrosine kinase inhibitor

Lung cancer is currently the leading cause of cancer mortality worldwide for both men and women, and the rate continues to increase. In 2018, there were 2.10 million new lung cancer cases, accounting for 11.6% of all cancer cases, and 1.80 million deaths from lung cancer, accounting for 18.8% of all cancer-related deaths.¹ In 2015, there were 733,000 new lung cancer cases and 610,000 lung cancer-related deaths in China.² Non-small cell lung cancer accounts for 80–85% of all lung cancer cases.³ Despite the improvements in chemotherapy, the 5-year survival rate of patients with advanced NSCLC is less than 3%.³ With the continuous development of new targeted drugs, the objective response rate (ORR) has been improved and the overall survival (OS) has also been prolonged.^{4–8} In addition, advances in gene detection technologies have led to the identification of oncogenes in lung cancers, such as aberrations in the HER2 gene. Human epidermal growth factor receptor 2 (HER2/ERBB2) is a receptor tyrosine kinase of the epidermal growth factor receptor (ErbB) family, which includes EGFR (HER1/ERBB1), HER3 (ERBB3) and HER4 (ERBB4).⁹ The HER2 gene (also known as erbB2 or neu) is located on the long arm of chromosome 17 (17q21) and is a proto-oncogene. HER2 is a type I transmembrane growth factor receptor tyrosine kinase consisting of 1255 amino acids with a relative molecular weight of 185,000 Da. The HER2 protein is composed of three functional domains: an extracellular ligand-binding domain (ECD), an α -helical transmembrane domain (TMD) and an

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intracellular tyrosine kinase domain (TKD). Ligand binding promotes receptor dimerization and autophosphorylation of the intracellular TKD, which induces HER2 kinase activity, resulting in the initiation of a variety of signaling pathways related to cell proliferation, differentiation and migration, such as the RAS/MAPK and PI3K/Akt pathways. No endogenous ligand has been identified for HER2 thus far. Several studies have shown that HER2 overexpression and/or gene amplification drive the occurrence and development of various cancers,¹⁰ such as breast cancer, ovarian cancer and gastric cancer. In breast cancer, HER2-targeted therapies including trastuzumab and pertuzumab have achieved significant efficacy and huge survival benefits, and these treatments are now considered standard of care.¹¹ However, HER2 alteration in NSCLC has different functional consequences compared with breast cancer¹² and is associated with poor disease prognosis.^{13,14} Definition of HER2 alterations in NSCLC is still integrated and the question remains whether all these alterations are relevant oncogenic driving factors. Therefore, the detection methods for HER2 and treatment targets for NSCLC are still urgent clinical problems in treating NSCLC patients. Currently, the FDA recommends immunohistochemistry (IHC), in situ hybridization (ISH) and fluorescence in situ hybridization (FISH) for detection of HER2 protein or gene amplification ([Supplementary Table 1](#)).

In this review, we discuss HER2 aberrations in NSCLC, including the diagnostic challenges and treatment strategies. Better understanding of the mutation types and mechanisms of HER2 mutants in NSCLC will facilitate the individualized treatment of such patients in the future.

HER2 Mutation in NSCLC

In 2004, the Cancer Genome Project and Collaborative Group reported the presence of HER2 mutations in 4.2% of lung cancer patients, with a rate of approximately 9.8% in lung adenocarcinoma patients.⁹ A number of studies have since reported that the HER2 mutation rate in NSCLC patients is 2%–6%, with higher incidences in Asians, never smokers, women and lung adenocarcinoma patients^{9,15–19} ([Supplementary Table 2](#)). These trends are similar to those observed with patients with EGFR mutation. In addition, several retrospective studies have shown that the most common type of HER2 mutation was in-frame insertions in exon 20.^{15,16,18,20}

Chen et al²¹ analyzed 54 tissue and blood samples of 48 patients with HER2 gene mutation or amplification and

found that 36 samples harbored HER2 gene mutation and 23 samples showed HER2 gene amplification. Li et al²² identified four cases of HER2 gene mutation without gene amplification and overexpression by FISH and IHC, respectively. These findings indicate that HER2 gene mutation has no significant correlation with protein expression and gene amplification suggesting that HER2 gene and protein aberrations may have different therapeutic and prognostic values.

As the use of next-generation sequencing (NGS) is constantly expanding, the number of identified HER2 mutations will continue to grow, especially in NSCLC. The NCCN guideline for NSCLC (2021.V2) recommended NGS testing to identify rare oncogenic driver variants for which effective therapy may be available. The Memorial Sloan Kettering Cancer Center reported that 83% of HER2 gene mutations detected by NGS occur in exons 18–21, which encode the TKD, and the most common mutation is a duplicated insertion of 12 base pairs (encoding YVMA) at codon 775 of exon 20, which is known as the HER2^{YVMA} subtype, p.Y772_A775dupYVMA (c.2324_2325ins12).^{15,23} Mutation in the TKD leads to conformational changes that result in narrowing of the ATP binding pocket, increasing HER2 kinase activity, and enhancing downstream signal transduction pathways that mediate cell proliferation, expansion and invasion and tumor development.^{18,23,24} A total of 31 subtypes of HER2 mutation were found that the predominant subtype was HER2^{YMVA} (42%), followed by G778_P780dup (9%), G776delinsVC (8%), S310F/Y (7%) and V776L (6%).²⁵ In addition, Sai-Hong et al^{26,27} found germline mutations in the HER2 transmembrane domain (G660D, V659E). Both mutations lead to increased stability of the wild-type protein and activation of the AKT pathway, indicating that the TMD mutations are carcinogenic and can lead to hereditary and sporadic lung cancer. In 2020, Wei et al²⁸ showed that non-TKD mutations accounted for 57.5% of HER2 mutant NSCLC cases. Among these mutations, a point mutation in exon 8 (S310F) was the most common, resulting in an altered kinase domain similar to that which was observed in the HER2^{YVMA} subtype.²⁹ The non-TKD mutations are considered potential targets for treatment. In our clinical practice, a middle-aged, never-smoking male patient with advanced lung adenocarcinoma had the HER2 S335C mutation. Partial response was achieved with a pan-HER inhibitor (pyrotinib), and the disease has been in remission for 10 months.³⁰

NGS analysis of HER2 co-mutations showed that 51.6–66% patients had TP53 gene mutation (mostly in exons 5–8), followed by mutations in LRP1B (18.2%), EPHA5 (9.1%), MLL3 (9.1%) and RB1 genes (8.0%).^{31,32} HER2 gene mutation and TP53 gene mutation were found to be poor prognostic factors for NSCLC.^{19,32}

Progress in HER2 Mutation–Targeted Therapy

Clinical trials have indicated the efficacy of small molecule tyrosine kinase inhibitors (TKIs) for HER2-mutated NSCLC; however, these studies are all Phase I–II trials using pan-HER TKIs with small sample sizes^{25,27,31,33–46} (Table 1). Compared with the efficacy of drugs direct to other NSCLC targets (EGFR, ALK, ROS-1), the ORR and progressive-free survival (PFS) of patients receiving HER2-targeted therapy are still insufficient.

Afatinib

Afatinib is a powerful and irreversible double inhibitor of EGFR and HER2 tyrosine kinase.³⁴ The ORR and disease control rate (DCR) in three independent cohorts were 13–19% and 70%, respectively.^{31,38,40} De Greve et al^{33,47} reported an ORR of afatinib in the treatment of HER2-mutated NSCLC of 3.8%–53.8%, and the PFS was 3.7–6.4 months. A retrospective analysis of 28 patients with HER2-mutated NSCLC treated with afatinib showed that the time of treatment failure was 2.9 months, and the duration of response (DoR) of the HER2^{YVMA} subtype was 9.6 months.⁴⁰ However, the response of patients with HER2 mutations to afatinib was different. Liu et al³¹ evaluated the clinical outcomes of 19 Chinese patients harboring ERBB2 20ins after afatinib treatment and found that patients with the G778_P780dup subtype achieved a longer median PFS (mPFS) and median OS (mOS) than patients with other 20ins (non-G778) subtypes (mPFS, 10 vs 3.3 months, P=0.32; mOS, 19.7 vs 7 months, P=0.16). Fang et al²⁵ found that the ORR and PFS of afatinib for patients with G778_P780dup (c.2331_2339dup) and G776delinsVC (c.2326delinsTTGT) that were treated with afatinib was 40% (4/10) and 7.6 months (95% CI 4.9–10.4); the ORR and PFS for the HER2^{YVMA} subtype was 0% (0/14) and 1.2 months (95% CI 0–2.4 months), and the ORR and PFS for HER2 missense mutation was 13% (1/8) and 3.6 months (95% CI 2.6–4.5). These results indicate that patients harboring G778_P780dup and G776delinsVC subtypes can achieve survival benefits from afatinib.

The above studies confirmed that patients with different subtypes of HER2 mutation have different sensitivities to afatinib, but the precise conclusions have been inconsistent. A prospective study with a large sample size is required to further clarify the therapeutic effect of afatinib on NSCLC harboring different HER2 mutant subtypes.

Neratinib

Neratinib is an irreversible TKI with an IC₅₀ of 59 nM for HER2. Nagano et al²³ reported that neratinib was more effective against the HER2^{YVMA} subtype in NSCLC cell lines, while NSCLC cells with L775P and L775S showed resistance. Studies in patient-derived organoids showed that neratinib monotherapy highly induced tumor cell death. Compared with neratinib alone, the combination therapy of neratinib + trastuzumab or neratinib + sirolimus showed enhanced anti-cancer effects, with the combination of neratinib + sirolimus showing the best effect.⁴⁸ The SUMMIT trial³⁶ showed that the ORR of neratinib was only 3.8% (1/26) in HER2-mutant NSCLC patients, but the mPFS was 5.5 months because 6 patients had been treated with neratinib for more than 1 year.

Osimertinib

Osimertinib was originally designed as a third-generation EGFR-TKI for NSCLC that acquired the TKI-resistant mutation T790M in EGFR. It was later found to have affinity for HER2 and it may be used as a monotherapy or combination therapy for HER2-mutated NSCLC. Early data suggested that although osimertinib was particularly effective against tumors with the EGFR T790M mutation, approximately 20% of EGFR T790M-negative patients also responded to osimertinib, suggesting that osimertinib may be effective for HER2-mutated NSCLC.⁴⁹ Shortly afterwards, Nagano et al²³ found that osimertinib demonstrated good efficacy against Ba/F3 cells expressing HER2 L755P and L755S mutations *in vitro*, which are the most common mutations in breast cancer, while it had a weak effect on cells harboring the HER2^{YVMA} mutation compared with afatinib and neratinib. Liu et al⁵⁰ reported the efficacy of osimertinib in the treatment of HER2-mutated NSCLC *in vitro*. An antitumor effect of osimertinib combined with the BET inhibitor JQ1 was observed, but there is no clinical trial data so far.⁵⁰

Poziotinib

Poziotinib is a newly developed TKI targeting HER2 with exon 20 mutation. It can overcome the conformational change of ATP-binding pocket caused by EGFR (HER1/

Table 1 Results of Clinical Data Targeting HER2 Aberrations Among Lung Cancer Patients Reported in Various Trials

Drug	Author(Year)	Trial	HER2 Aberrations	No. Patients	ORR (%)	mPFS (m)	mOS (m)	DoR (m)	Sensitive HER2 Subtype
Afatinib	De Greve et al (2015) ³⁶	Retrospective	HER mutation	7	0	4.25	NA	4	NA
	Ou et al (2017) ²⁷	Retrospective	HER2 mutation	15	27(4/15)	NA	NA	NA	NA
	Liu Z et al (2016) ³¹	Retrospective	HER2 ex20ins	19	15.8(3/19)	4.5	11.5	NA	HER2 ^{G778_P780dup}
	Peters S et al (2018) ³⁵	Retrospective	HER2 ex20ins	28	19 (3/16)	NA	NA	9.6	HER2 ^{YVMA}
	Dziedziszko R et al (2019) ³³	Phase II trial	HER2 ex20ins	13	7.7(1/13)	3.7	13.1	NA	HER2 ^{YVMA}
	Fang W et al (2019) ²⁵	Retrospective	HER2 ex20ins	32	15.6(5/32)	3.2	NA	NA	HER2 ^{G778_P780dup} , HER2 ^{G776delinsVC}
	Lai et al (2018) ³⁴	Retrospective	HER2 ex20ins, HER2 ^{V659E} (ex17) HER2 ^{S310F} (ex8)	27	13(3/23)	NA	7	3	HER2ex20ins
Neratinib	Hyman DM et al (2018) ³⁹	Phase II trial	HER2 ex20ins HER2 ^{S310F} (ex8) HER2 ^{L755S} (ex19)	26	3.8(1/26)	5.5	NA	NA	HER2ex20ins
	Robichaux et al (2018) ⁴²	Phase I/II trial	HER2 ex20ins	12	42(5/12)	5.6	NA	4.6	HER2 ^{YVMA} HER2 ^{G778_P780dup}
Dacomitinib	Kris et al(2015) ⁴⁴	Phase II trial	HER2 ex20ins	26	12(3/26)	4	9	NA	HER2 ^{G778_P780dup}
			HER2 amplification	4	0	1,1,5,5	5,7,15,22	NA	NA
Pyrotinib	Wang Y et al (2019) ⁴⁵	Phase II trial	HER2 ex20ins	15	53.3	6.4	12.9	7.2	HER2ex20ins
	Zhou C et al (2020) ⁴⁶	Phase II trial	HER2 ex20ins	60	31.7(19/60)	NA	NA	1.0	HER2ex20ins
TAK-778 (Mobocertinib)	Gonzalez F et al(2020) (AACR 2020)		EGFR ex20ins HER2 ex20ins	60	34.78(21/60)	NA	NA	NA	NA
	Hainsworth et al (2018) ⁵⁰	Phase IIa trial	HER2 mutation	14	21(3/14)	NA	NA	NA	NA
Trastuzumab +Pertuzumab			HER2 overexpress or amplification	16	13(2/16)	NA	NA	NA	NA

T-DMI	Peters S et al (2019) ⁸²	Phase II trial	HER overexpression 2+	29	0	2.6	12.2	NA	NA
	Li BT et al (2018) ⁵²	Phase II trial	HER overexpression 3+	20	20%	2.7	12.1	NA	NA
DS8201	Li BT et al (2018) ⁵²	Phase II trial	HER2 mutations	18	44(8/18)	5	NA	4	NA
	Tsurutani J et al(2020) ⁵⁵	Phase I trial	HER2 mutations	11	88.2	14.1	NA	NA	NA
	Smit EF et al (2020) ⁵⁶	Phase II trial	HER2 overexpression (2+ or 3+)	18	58.5	14.1	NA	NA	NA
			HER2 mutations	42	61.9	14	NA	NA	NA

ErbB1) and HER2 exon 20 mutation and has inhibitory effects on the EGFR/HER2/HER4 signaling pathway.⁴² Therefore, poziotinib is considered effective as an inhibitor of HER2 with the exon 20 mutation. In a preclinical trial of cell line- and patient-derived xenotransplantation models (PDXs), poziotinib inhibited EGFR, HER2 and HER4 more effectively than other pan-HER TKIs.⁴² In a Phase II clinical trial of poziotinib (16 mg daily) for NSCLC with HER2 exon 20 mutation (12 cases), the ORR reached 42% and the mPFS was 5.6 months.⁵¹ However, 60% of patients developed rash and diarrhea, and one case had drug-related pneumonia. In another study, mice harboring HER2^{YVMA} were treated with poziotinib combined with trastuzumab emtansine (T-DM1), and the average tumor shrinkage rate was 47% compared with controls.⁴² Poziotinib is currently a potential drug to target the HER2 exon 20 mutation.

Dacomtinib

Dacomtinib is an irreversible pan-HER-TKI that targets EGFR, HER2 and HER4. A small sample phase II clinical study was designed to assess the efficacy and safety of dacomtinib therapy against HER2 aberrations in NSCLC.³⁷ The trial included 30 patients (including 26 with HER2 mutations and 4 with HER2 gene amplification). The ORR of the overall patient group was only 10%, while the ORR of the patients with gene amplification was 0, and only three patients reached PR, including two cases HER2^{P780_P781insGSP} and one case with HER2^{M774 delinsWLV}, indicating that dacomtinib had poor effects on HER2 YVMA subtypes.

Pyrotinib

Pyrotinib is a class of irreversible pan-HER TKIs developed by China. In vitro and in vivo test results showed that the antitumor activity of pyrotinib was significantly better than that of afatinib and T-DM1 in organoids and PDXs harboring the HER2^{YVMA} mutant subtype.⁴⁵ The concentration of pyrotinib in animal blood was 10 times higher than afatinib, and the tumor shrinkage rate was 52.2%. In 2020, Zhou et al⁴⁶ reported a single arm, open label, multicenter phase II clinical study of pyrotinib for the treatment of HER2-mutated NSCLC as second-line or above treatment, and the ORR was 31.7% (95% CI 18.8–43.2%) in 60 patients with stage IIIB and IV NSCLC with HER2 exon 20 mutation. The ORR was similar in different subtypes of NSCLC. The mDoR was 6.9 months (95% CI 4.9–11.1 months), the mPFS was 6.8 months (95% CI 5.5–

8.3 months), and the mOS was 14.4 months (95% CI 12.3–21.3 months). Pyrotinib is expected to improve the outcome of NSCLC patients with HER2 exon 20 mutation.

TAK-778 (Mobocertinib)

Mobocertinib is a research-based oral EGFR/HER2 inhibitor for the treatment of NSCLC with EGFR/HER2 mutation, including the EGFR exon 20 insertion mutation (EGFR ex20ins). In the dose exploration study (NCT02716116), the ORR of the total population was 34.78% (EGFR ex20ins and HER2 ex20ins), and the standard dose was determined to be 160 mg daily. In the follow-up cohort expansion study (AACR 2020), an ORR of only 43% was published for EGFRex20ins NSCLC patients, and the mPFS was 7.3 months. Therefore, the efficacy of TAK-778 in refractory NSCLC patients with EGFR ex20ins seems to be better than other second-line treatment schemes in the real world. However, the efficacy of HER2ex20ins remains to be determined.

Tucatinib (Tukysa)

Tucatinib is a small oral TKI with high selectivity to the HER2 TKD. HER2 CLIMB research⁵² showed that in the comparison of the combination of tucatinib plus trastuzumab and capecitabine with placebo plus trastuzumab and capecitabine to treat HER2-positive breast cancer patients, the 1-year PFS rate was doubled (33.1% vs 12.3%, $p < 0.001$) and PFS (7.8 months vs 5.6 months, $p < 0.001$) and OS (21.9 months vs 17.4 months, $p=0.005$) were significantly longer in the combination treatment group. It is worth noting that tucatinib also has good brain entry activity. The triple-drug therapy reduces the risk of disease progression or death in patients with brain metastases by 52%, and thus has a significant effect on patients with brain metastases. In 2020, FDA approved this regimen for locally advanced unresectable or metastatic HER2-positive breast cancer, including patients with brain metastases who had previously received anti-HER2 regimens, individually or in combination. However, whether tucatinib is effective against HER2-mutated NSCLC has not been determined.

Monoclonal Antibodies and Antibody-Coupled Drugs

Trastuzumab

Trastuzumab is an IgG2 monoclonal antibody that targets the HER2 extracellular domain. Trastuzumab binds with

HER2 extracellular domain region IV, blocks signal transduction, inhibits HER2 activity and promotes the immune system to recognize and kill tumor cells.⁵³ A retrospective cohort study (EUHER2 study)⁵⁴ showed that chemotherapy combined with trastuzumab was effective against HER2 mutant NSCLC, with a response rate as high as 50%. However, no study has compared trastuzumab with platinum containing dual drug chemotherapy.

Pertuzumab

Pertuzumab is another humanized monoclonal antibody against HER2 that binds to the extracellular domain II of HER2, inhibits the formation of heterodimer complexes between HER2 and other receptors, and inhibits the downstream signal transduction pathway. Hainsworth et al³⁵ recruited 14 patients with NSCLC harboring HER2 mutation undergoing trastuzumab combined with pertuzumab (the MyPathway basket trial) and the ORR was 21% (3/14).

Trastuzumab Emtansine (T-DM1)

Trastuzumab emtansine is a type of antibody-drug conjugate (ADC) composed of the HER2 monoclonal antibody trastuzumab and emtansine (T-DM1), an inhibitor of microtubule aggregation. Degradation of the T-DM1 complex in lysosome results in the release of cytotoxic anti-tubule agents in HER2-positive tumor cells. In phase II clinical trials of T-DM1 treatment for second-line and above HER2-altered NSCLC⁵⁵ (including mutations, gene amplification, and protein expression), the ORR reached 6.7%, and the ORR in the HER2 mutant NSCLC subgroup achieved 14.3%. In another phase II basket trial,³⁹ T-DM1 monotherapy (3.6 mg/kg) was given to 18 patients with advanced HER2-mutant lung adenocarcinomas; the ORR was 44%, the mDoR reached 4 months (range 2–9 months), and the mPFS was 5 months (95% CI 3–9 months). The adverse reactions were mild, mainly including infusion reaction and myelosuppression. Based on the results of the T-DM1 trial, NCCN guidelines (2021. V2) recommend T-DM1 for the treatment of HER2-mutated NSCLC.⁵⁶

Trastuzumab Deruxtecan (Enhertu, T-DXd, DS-8201)

Trastuzumab deruxtecan is a novel ADC that is composed of the humanized anti-HER2 antibody trastuzumab with the topoisomerase I inhibitor deruxtecan. The ratio of chemotherapy drug to antibody reaches approximately 7–8, which makes it effective in tumors with low HER2 expression.⁵⁷ A Phase I clinical study (DS-8201-a-J101)⁴⁴

showed that the ORR was 72.7% (8/11) and mPFS was 11.3 months (95% CI 8.1–14.3). In 2020, the American Society for Clinical Oncology (ASCO) released the interim analysis results of phase II clinical trials (Destiny-Lung-01)⁴³ that showed that ORR was 61.9% and the mPFS was 14 months. DoR and OS data were not yet available. Stratification analysis based on HER2 mutation and protein expression (IHC 2+ or 3+) showed no significant difference in efficacy among groups. The incidence of drug-related adverse reactions was 52.4% in patients with grade 3 tumors or above. Myelosuppression (neutropenia and anemia) was common. Approximately 11.9% of patients developed grade 2 drug-related interstitial pneumonia. DS-8201 showed a significant effect on HER2-altered advanced NSCLC and was recommended by NCCN guidelines (2021.V2).

Immune Checkpoint Inhibitors (ICIs)

ICIs, such as PD-1 inhibitors, PD-L1 inhibitors and CTLA-4 inhibitors, are drugs that unleash an immune system attack on cancer cells. Immunotherapy is currently approved by the FDA for treatment of lung cancer. A retrospective analysis was performed on the efficacy of immunotherapy in patients with driver gene-positive advanced NSCLC published by the European Association of Oncology (ESMO) in 2019.⁵⁸ The results showed that the mPFS of HER2 mutation NSCLC was 2.5 months (95% CI 1.8–3.5 months); the 6-month and 12-month PFS rates were 22.7% and 13.6%, respectively; and the mOS was 20.3 months. Similar to NSCLC patients with EGFR mutation and ALK gene arrangement, HER2-mutated NSCLC patients do not benefit from immunotherapy. Lin et al⁵⁹ suggested that the tumor microenvironment lacking CD8+ T cell infiltration may explain the poor efficacy of ICIs in the treatment of EGFR and HER2-driven NSCLC.

Secondary HER2 Mutation

HER2 mutation occurs in some EGFR-mutated NSCLC patients during EGFR-TKI treatment, and these mutations in HER2 are considered to be one of the acquired resistance mechanisms against EGFR-TKI drugs. In vitro experiments indicated that mutant HER2 forms heterodimers through HER2 and EGFR, resulting in phosphorylation of the intracellular tyrosine kinase and activation of downstream signal transduction pathways, leading to cell survival, invasion and tumorigenicity and eventual EGFR-TKI resistance.²⁴ Hsu et al⁶⁰ found that HER2 D16 is

a new mechanism of osimertinib resistance independent of the SRC pathway. In the AURA study,⁶¹ one patient acquired HER2 mutation (HER2^{YVMA}) during osimertinib treatment and was subsequently sensitive to T-DM1.

HER2 Gene Amplification

Compared with breast cancer, HER2 amplification is rare in NSCLC, with an incidence rate of approximately 2–4% in tumor samples.^{18,62} Fluorescence in situ hybridization (FISH) is the standard method for detection of HER2 gene amplification and shows high specificity, standardization and consistency in breast cancer.^{63,64} HER2 amplification is usually defined as HER2/CEP17 \geq 2.0 (FISH testing by dual probe assay). If the number of chromosome 17 doubles in a single cell, the number of copies of HER-2/neu per cell is relatively increased (because of polyploidization of chromosome 17) or the activity of upstream promoter increased, which led to large expression of HER2 gene.¹⁴ The 2018 CAP/ASCO guideline recommendations for HER2 positivity criteria is an absolute HER2 gene copy number \geq 6/cell or HER2/CEP17 was $<$ 2. Whether high copy number of HER2 has a prognostic or predictive value is unclear.^{22,65} Future studies should distinguish between individuals with HER2 gene amplification and individuals with high HER2 copy number increase but without amplification. We are also exploring other methods to quantify HER2 gene amplification (mRNA level), such as quantitative polymerase chain reaction and real-time quantitative reverse transcriptase-polymerase chain reaction.⁶⁶

HER2 Gene Amplification Therapy

Lapatinib is a dual TKI that blocks EGFR and HER2 tyrosine kinase activity by binding to the ATP-binding site of the receptor's intracellular domain. In a randomized phase II study,⁶⁷ lapatinib reduced the tumor size by 51% in two patients with HER2-amplified NSCLC. In 2005, Clamon and others proved that trastuzumab alone could not achieve clinical benefits in the treatment of NSCLC.⁶³ Interestingly, in 2006, Cappuzzo first reported the case of a 60-year-old female nonsmoker with increased copy numbers of EGFR and HER2 genes by FISH who responded to trastuzumab.⁶⁸ DNA sequencing later performed on the same tissue detected an EGFR exon 21 mutation (A859T) and a HER2 exon 20 mutation (G776L). MyPathway, an open-label, phase IIa multiple basket study,³⁵ reported that in patients with HER-positive metastatic NSCLC (IHC3+ or HER2/CEP17 \geq 2 or copy number \geq 6), trastuzumab combined with pertuzumab had

limited efficacy (ORR=13%). Li et al³⁹ reported that the DCR of T-DM1 in 18 patients with HER2 mutation (NGS copy number increase or HER2/CEP17 > 2) was only 11% in a phase II study, and the mPFS was only 5 months.

Acquired HER2 Gene Amplification

In 2005, Cappuzzo et al⁶⁸ reported that increased copy number of the HER2 gene in EGFR-positive NSCLC patients was associated with gefitinib sensitivity, supporting the use of HER2 FISH analysis for selection of patients for TKI therapy. HER2 amplification is one of the main mechanisms of EGFR-TKI acquired resistance in patients with EGFR+ NSCLC.^{69,70} In 2011, Yonesaka et al⁷¹ showed that activation of ERBB2 signaling in cell lines through ERBB2 amplification led to persistent extracellular signal-regulated kinase 1/2 signaling and consequently to cetuximab resistance, suggesting that ERBB2 inhibitors in combination with cetuximab may be a therapeutic strategy in patients with cetuximab-resistant cancers. In a preclinical study,⁶⁹ the rate of HER2 amplification in EGFR-TKI-resistant NSCLC cells was found to be 12%. This mechanism of drug resistance has also been confirmed in clinical studies and later confirmed in re-biopsy of patients with NSCLC resistant to EGFR-TKIs, showing that HER2 gene amplification was about 13%.⁷² In addition, HER2 amplification was found in 1 of 9 EGFR T790M-NSCLC patients with primary resistance to osimertinib, suggesting that HER2 amplification and EGFR T790M mutation may be mutually exclusive.^{73,74} Although HER2 gene amplification may be a mechanism for EGFR-TKI resistance, it is necessary to distinguish between HER2 gene amplification, chromosome 17 numbers and co-mutations. Existing data has suggested that only high levels of HER2 amplification can drive tumor resistance, just as MET-dependent acquired gefitinib resistance occurs only in the presence of high levels of MET gene amplification (> 12 gene copies) and rarely occurs without pharmacological pressure.⁶⁸

As outlined above, in the context of acquired drug resistance, clinical research should distinguish among the mechanisms leading to drug resistance, such as a high level of HER2 gene amplification driving tumor resistance, any level of HER2 amplification and the presence of EGFR mutation coexisting concomitant events. The high level of HER2 gene amplification is one of the reasons for the resistance of colorectal cancer to cetuximab, an EGFR monoclonal antibody.⁷⁵

HER2 Overexpression

At present, there is no consensus on HER2 protein expression score and diagnostic criteria in NSCLC.⁷⁶ There are currently two scoring systems for HER2 protein expression, the IHC scoring system and H-SCORE. The incidence of HER2 (IHC \geq 2+) in NSCLC is approximately 24% and that of HER2 (IHC3+) is approximately 3–10%.^{77,78} H-SCORE, a semi-quantitative assessment performed by multiplying staining intensity with the percentage of positive tumor cells, ranges from 0 to 300 and the results are classified as high (\geq 200), intermediate (\geq 100–200) and negative (<100). The consistency among the different methods for HER2 expression assessment is poor, perhaps resulting in the heterogeneity in the positive rates of HER2 overexpression among different papers. The IHC scoring system for HER2 expression is currently the most common method of detection in pathological diagnosis in clinical practice.

Bunn et al⁷⁹ found some correlation between the expression of HER2 protein on the cell surface evaluated by IHC and the increase of HER2 copy number evaluated by FISH in NSCLC cell lines.

HER2 Protein Overexpression Therapy

Although trastuzumab showed antitumor activity in NSCLC cell lines with HER2 overexpression in vitro, no clinical benefit was found in patients with IHC 2+ or 3+ NSCLC treated with trastuzumab alone.⁶³ In addition to gemcitabine combined with cisplatin, trastuzumab was added to 103 patients with IHC 2+ or 3+ NSCLC; compared with chemotherapy alone, the mPFS of the patients treated with the combination therapy was 8.5 months.¹² A phase II clinical study initiated by the Eastern Cancer Cooperation Group in 2004⁸⁰ to evaluate the efficacy of paclitaxel and carboplatin combined with trastuzumab in the treatment of NSCLC with HER2 overexpression (IHC 1+ to 3+), including IHC 1+ (38/139; 27%), IHC 2+ (31/139; 22%), and IHC 3+ (13/139; 9%), the survival rate of the combination group was similar to that of the chemotherapy group (mPFS, 3.3 months). Similarly, in 38 patients with NSCLC with HER2 overexpression, trastuzumab combined with pertuzumab had similar efficacy to chemotherapy, and there was no significant correlation between HER2 protein expression levels and PFS.^{81,82} Hotta et al⁵⁵ found that among NSCLC patients with IHC 2+ or 3+ treated with T-DM1, the ORR was only 6.7% and the mPFS was 2.0 months. In further study on

the effect of T-DM1 on patients with different HER2 protein expression levels,⁴¹ the ORR of the IHC 1+ cohort was 0% and that of the IHC 3+ cohort was 20%. The PFS and OS of the two groups were similar. Further analysis of co-mutations showed that 75% of patients in the IHC 3+ cohort harbored HER2 gene amplification. Therefore, HER2 protein level could not predict the effect of T-DM1. DS-8201 has strong antitumor activity in HER2-overexpressing NSCLC mice.⁵⁷ Interim data analysis of the Destiny-Lung01 clinical study in 2020⁴³ indicated that the therapeutic effect was significant in HER2-overexpressing metastatic NSCLC with a confirmed ORR of 24.5%, and there was no apparent difference in ORR according to HER2 expression (IHC 3+ vs 2+).

Acquired HER2 Overexpression

HER2 protein overexpression is found in EGFR+ NSCLC patients, with an incidence of approximately 10%, and considered as a potential driving factor of EGFR-TKI resistance.⁶⁹ De Langen et al⁸³ found that HER2 protein expression level increased over time during EGFR-TKI treatment (median HER2 IHC 2+, H-score 100; median HER2 IHC 3+, H-score 240). These findings suggest that the selective pressure from long-term exposure to EGFR-TKIs may lead to the amplification of existing clones carrying specific gene changes; these clones may become dominant, leading to drug resistance to targeted drugs.^{83–85}

An in vitro experiment⁸⁴ suggested that T-DM1 may inhibit resistance in EGFR+ NSCLC by targeting the HER2 pathway. De Langen et al⁸³ showed that in T-DM1-treated patients with acquired HER2 overexpression, the ORR was 46% (11/24) and the DoR was 5.6 months. Stratified analysis showed that the effective rate was significantly improved in patients with IHC 3+ (12 patients, ORR 67%) or HER2 copy number ≥ 10 (4 patients, ORR 100%); among cases with T-DM1 resistance, 66.7% (4/6) of patients showed negative HER2, indicating that T-DM1 targeted HER2 protein overexpression. Osimertinib combined with T-DM1 can improve the efficacy of osimertinib by delaying the drug resistance of EGFR+ NSCLC cell lines. The effect of HER2 protein expression was studied in PC9/HER2c1 xenotransplantation models, and the results showed that T-DM1 combined with osimertinib inhibited the growth of tumor cells and delayed drug resistance to osimertinib in some cell lines or tissues.^{77,78,86} These data suggested that EGFR-TKIs combined with ADC (T-DM1) may be an effective treatment strategy for EGFR+ NSCLC, with the main purpose of

delaying the emergence of drug resistance. However, this idea is still in the preclinical research stage, and there are no in vivo data available.

Conclusion

The discovery of cancer-driving gene mutations in NSCLC, such as EGFR gene mutation, ALK gene rearrangement, ROS-1 gene rearrangement and others, has allowed for the development of molecular-targeted lung cancer therapies. NGS has become a standard diagnosis and treatment process for NSCLC patients and many potential targets have been found. HER2-positive NSCLC has gradually attracted clinical attention and is regarded as a unique molecular subtype of NSCLC. Whether co-mutation (for example, TP53 mutation) and background (primary and acquired drug resistance) and individualized treatment for patients may be the direction of future research.

Compared with the role of HER2 in other HER2-positive tumors (breast cancer and gastric cancer), HER2 mutation in NSCLC is considered to be more important than protein overexpression or gene amplification in the development and treatment of NSCLC. At present, many clinical studies are focused on treatments for HER2-positive NSCLC, including pan-HER TKIs, monoclonal antibodies, and ADCs, but they are limited to phase II clinical studies with small samples. So far, T-DM1 and DS-8201 have been recommended by NCCN guidelines.

Acquired HER2 mutation and HER2 gene amplification are considered to be the main mechanisms leading to EGFR-TKI resistance.^{24,49,62} HER2 overexpression is a potential factor contributing to EGFR-TKI resistance.^{55,77,87} There are acquired changes in HER2, and the efficacy of targeting HER2 drugs is still under further exploration.

Effective therapeutics and targets for NSCLC with HER2 aberration remain to be identified. Identification of the type of HER2 aberration in NSCLC (gene mutation, gene amplification, protein expression) and evaluation of the sensitivity of different drugs to HER2 aberrations and HER2 mutation subtypes (non-TKD region, HER2ex20ins, HER2^{YVMA}) will be critical. Future treatment strategies for NSCLC should be developed according to the types of HER2 aberrations.

Abbreviations

DCR, Disease control rate; DoR, Duration of response; EGFR, Epidermal growth factor receptor; ECD,

Extracellular ligand-binding domain; ICI, Immune checkpoint inhibitor; IHC, Immunohistochemistry; NSCLC, Non-small cell lung cancer; NGS, Next generation sequencing; OS, Overall survival; PFS, Progression-free survival; PDX, Patient-derived xenotransplantation model; TKD, Tyrosine kinase domain.

Disclosure

The authors report no conflicts of interests in this work.

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