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REVIEW

Consensus for HER2 alterations testing in non-small-cell lung cancer

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Human epidermal growth factor receptor 2 (HER2) is a transmembrane glycoprotein receptor with intracellular tyrosine kinase activity. Its alterations, including mutation, amplification and overexpression, could result in oncogenic potential and have been detected in many cancers such as non-small-cell lung cancer (NSCLC). Such alterations are, in general, considered markers of poor prognosis. Anti-*HER2* antibody-drug conjugates, e.g. trastuzumab deruxtecan (T-DXd, DS-8201) and disitamab vedotin (RC48), were recently approved for *HER2*-positive breast and gastric cancers. Meanwhile, several *HER2*-targeted drugs, such as T-DXd, neratinib, afatinib, poziotinib and pyrotinib, have been evaluated in patients with advanced NSCLC, with several of them demonstrating clinical benefit. Therefore, identifying *HER2* alterations is pivotal for NSCLC patients to benefit from these targeted therapies. Recent guidelines on *HER2* testing were developed for breast and gastric cancer, however, and have not been fully established for NSCLC. The expert group here reached a consensus on *HER2* alteration testing in NSCLC with the focus on clinicopathologic characteristics, therapies, detection methods and diagnostic criteria for *HER2*-altered NSCLC patients. We hope this consensus could improve the clinical management of NSCLC patients with *HER2* alterations.

Key words: non-small-cell lung cancer, human epidermal growth factor receptor 2, gene testing, amplification, mutation, overexpression

INTRODUCTION

With the discovery of oncogenic drivers and the approval of tyrosine kinase inhibitors (TKIs) targeting these drivers, the treatment strategy for advanced non-small-cell lung cancer (NSCLC) has moved from pathological-based to molecular-based modalities. These advances have made companion diagnostics for NSCLC a new standard in clinical

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decision-making, including human epidermal growth factor receptor 2 (HER2, also known as ErbB2) alterations. *HER2* alterations, a well-recognized mediator of the carcinogenic process in a wide range of solid tumors, mainly include *HER2* mutation, *HER2* amplification and *HER2* overexpression, with incidence rates of 1%-6.7%, 2%-22% and 7.7%-23%, respectively, in NSCLC, and all of them were associated with poor prognosis.[1-5]

In 2019, the United States Food and Drug Administration (FDA) issued an accelerated approval of trastuzumab deruxtecan (T-DXd, DS-8201), a novel *HER2* antibody-conjugated drug (ADC), for patients with unresectable or metastatic *HER2*-positive breast cancer (BC) administered two or more prior anti-*HER2*-based treatments. It was also approved in 2021 for locally advanced or metastatic *HER2*-positive gastric or gastroesophageal adenocarcinoma with failure on trastuzumab-based therapy. Another novel ADC, disitamab vedotin (RC48), has demonstrated a clinically meaningful response and survival benefit in patients with *HER2*-overexpressing [immunohistochemistry (IHC) 2+ or 3+] gastric or gastroesophageal junction cancers administered ≥2 prior lines of systemic treatments, with an overall response rate (ORR) of 18.1% and a median progression-free survival (mPFS) of 3.8 months.[6] On the basis of these findings, RC48 was approved conditionally by the National Medical Products Administration of China. Recently, several anti-*HER2*-targeted drugs, such as T-DXd, ado-trastuzumab emtansine (T-DM1) and the pan-HER TKIs afatinib, neratinib, poziotinib or pyrotinib, have shown effectiveness in NSCLC with *HER2* alterations, with an ORR of 3.8%-55% and an mPFS of 3.0-8.2 months.[7,8] Accordingly, testing *HER2* alterations, especially *HER2* mutation, amplification and protein expression as companion diagnostics, attracts increasing attention in the treatment and prognosis of NSCLC.[9] Current guidelines on *HER2* testing, however, were largely developed for breast or gastric cancer, and its criteria in NSCLC is still lacking.

This consensus was formed according to a Delphi method. The expert group had several rounds of deep discussion and voted on clinical issues related to *HER2* alterations testing in NSCLC. A consensus was reached when at least 60% of the experts voted for agreement. This consensus summarized the epidemiological and clinicopathologic characteristics and recommended therapies for *HER2*-altered NSCLC; it also explored the diagnostic technologies for assessing *HER2* alterations in NSCLC, and proposed possible research directions. The recommendations contained in this consensus were based on the current evidence and the clinical experience of the expert panel. We believe this timely consensus would be valuable for clinical practice as well as research in this field.

CONSENSUS ON *HER2* ALTERATIONS IN NSCLC

Epidemiology of HER2 alterations in NSCLC

HER2, also known as ErbB2, is one of the ErbB family of proteins that include epidermal growth factor receptor (EGFR or HER1/ErbB1), ErbB2, EGFR3 (or HER3/ErbB3), and EGFR4 (or HER4/ErbB4).[10] While capable of homodimerization,[11] *HER2* favors heterodimerization with other ErbB family members (EGFR, HER3 or HER4) when they are bound to ligands.[12,13] Dimerization of *HER2* receptor activates downstream cascades, including primarily the phosphatidylinositol-3-kinase/protein kinase B (AKT) and mitogen-activated protein kinases (MAPK) signaling pathways, which are indispensable in cell proliferation, differentiation and migration.[14] Moreover, *HER2* mutations and amplification are some of the mechanisms of acquired resistance to EGFR TKIs in NSCLC patients.[15,16]

The frequencies of *HER2* alterations, including *HER2* mutation, *HER2* amplification and protein overexpression, in NSCLC are shown in Table 1. *HER2* mutations were firstly reported in 2004 to be present in 4.2% (5/120) of unselected NSCLC cases, and in 9.8% (5/51) of lung adenocarcinoma patients.[17] Subsequently, increasing evidence confirms the presence of *HER2* mutations in NSCLC at a frequency of ~1%-3% among European and American populations, and 1.4%-6.7% in the Asian population (Table 1). Although most studies do not detail the use of previous targeted treatments, it was reported that *HER2* mutations are acquired in 1% of EGFR TKI-treated patients.[15] The *HER2* gene is a proto-oncogene located at 17q11.2-q12, which encodes a transmembrane glycoprotein with intrinsic tyrosine kinase activity. *HER2* belongs to the classical superfamily of receptor tyrosine kinases, which have extracellular, transmembrane and intracellular domains.[18] *HER2* mutations mainly occur in the intracellular domain, with the most common types being in-frame insertion mutations in exon 20 (48%), including *A775_G776insYVMA* (33.9%), *G776delinsVC* (5.7%) and *G778_P780insGSP* (3.4%) mutations; other frequent *HER2* mutations include *E1021Q*, *A1232FS* (1.2%) and *A1057V* (1.7%) in exons 22-31 and *I655V* (4.5%) in the transmembrane domain, as well as *S310F* (5.1%), *P122L* (2.3%) and *G222C* (1.1%) in the extracellular domain.[19]

HER2 copy-number amplification was demonstrated in 2%-22% NSCLC and *HER2* protein overexpression in 7.7%-23% based on different methods and patient populations (Table 1). *HER2* amplification was reported in 2% of first-line osimertinib-treated NSCLC patients who experienced disease progression and/or discontinued treatment, compared with 5% in second-line osimertinib-treated NSCLC patients.[15,16] Contrary to observations in BC, the association between *HER2* amplification and *HER2* expression in NSCLC is poor.[2] Only a limited subset of reported cases had both *HER2* mutation, *HER2* amplification and/or *HER2* expression in lung cancer (LC), suggesting that each of these abnormality types may represent distinct clinical entities, clinicopathologic features and therapeutic targets.[5,17,20,21]

Clinicopathologic features of HER2 alterations in NSCLC

NSCLC with *HER2* mutations are more likely to be found in adenocarcinoma or adenosquamous carcinoma, never (less than 100 cigarettes in a life time) or mild smokers (More than 100 cigarettes and less than 100 cigarette-years in a

Table 1. Incidence rates of HER2 alterations in NSCLC by country or regions

Country or region	Patients and specimens	Methods for evaluating HER2 alterations	Incidence rates of HER2 alterations		
			HER2 mutation	*HER2* amplification	HER2 overexpression
USA	Stage IV or recurrent lung adenocarcinoma cases administered no targeted therapy; tissue specimens[5]	• Mutations assessed by fragment analysis, mass spectrometry genotyping and Sanger sequencing • Amplification assessed by FISH (HER2/CEP17 ≥2.0) • HER2 overexpression assessed by IHC (3+/2+)	3% (4/148)	3% (5/175)	0 (0/25)
	Metastatic or recurrent lung adenocarcinoma; tissue specimens[1]	• NGS or Sanger sequencing	2.6% (24/920)	NA	NA
Australia	Primary NSCLC cases administered curative intent surgical resections; tissue specimens[10]	• Sanger sequencing	1% (1/100)	NA	NA
Europe	NSCLC; tissue specimens[90]	• Direct DNA sequencing	1.7% (65/3800)	NA	NA
Germany	Advanced and/or metastatic stage IIIB and IV NSCLC; tissue specimens[84]	• Amplification assessed by FISH (HER2/CEP 17 >2.0) • HER2 expression assessed by IHC (3+/2+)	NA	2% (7/378)	20% (83/410)
Italy	NSCLC patients administered surgical resection; tissue specimens[91]	• Amplification assessed by FISH (HER2/CEP 17 >2.0) • HER2 expression assessed by IHC (3+/2+)	NA	22% (9/41)	23% (26/115)
	Lung adenocarcinoma cases administered surgical resection; tissue specimens[62]	• PCR-single-strand conformational polymorphism	2.2% (9/403)	NA	NA
China	Wild-type EGFR lung adenocarcinoma patients administered no preoperative neoadjuvant therapy; tissue specimens[85]	• Mutations assessed by direct DNA sequencing • HER2 expression assessed by IHC (3+/2+)	4.8% (22/456)	NA	15.4% (55/357)
	NSCLC; tissue specimens or ctDNA[92]	• NGS	3.0% (NA/16 015)	1.7% (NA/16 015)	NA
	Primary NSCLC (Taiwan) cases administered curative intent surgical resections; tissue specimens[10]	• Sanger sequencing	1.4% (2/145)	NA	NA
	Lung adenocarcinoma patients administered no neoadjuvant treatment; tissue specimens[93]	• Direct DNA sequencing	3.57% (8/224)	NA	NA
	NSCLC cases administered surgical resection; tissue specimens[22]	• Direct DNA sequencing	1.9% (35/1875)	NA	NA
	NSCLC cases administered no chemotherapy or radiotherapy; tissue specimens[94]	• Sanger sequencing	2.4% (21/859)	NA	NA
Japan	Primary NSCLC cases administered surgical resection with curative intent; tissue specimens[10]	• Sanger sequencing	3% (8/269)	NA	NA
	Lung cancer patients administered pulmonary resection	• Direct DNA sequencing	2.6% (13/504)	NA	NA
Korea	Patients initially diagnosed with metastatic NSCLC; tissue specimens[29]	• NGS	2.0% (22/1108)	1.4% (15/1108)	NA
	NSCLC patients administered surgical resection; tissue specimens[2]	• Mutations assessed by direct DNA sequencing • Amplification assessed by FISH (HER2/CEP 17 >2.0) • HER2 expression assessed by IHC (3+/2+)	6.7% (7/104)	14.3% (46/321)	7.7% (25/321)
India	NSCLC; tissue specimens[95]	• Direct DNA sequencing	1.5 % (3/204)	NA	NA

CEP17, chromosome 17 centromere; ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; NA, not available; NGS, next-generation sequencing; NSCLC, non-small-cell lung cancer.

life time) and females. Shigematsu et al.[10] reported that *HER2* mutations predominantly occur in adenocarcinoma compared with other histological subtypes (2.8% versus 0%, $P = 0.02$) and in never smokers compared with smokers (More than 100 cigarettes in a life time) (2.8% versus 0%, $P = 0.02$). Similarly, Tomizawa et al.[3] reported a frequency of *HER2* mutations of 2.6% (13/504) in LC, versus 14.1% (11/78) in the subgroup of never smokers with adenocarcinoma or adenosquamous cell carcinoma without *EGFR* mutations. Compared with *EGFR* and *HER2* double wildtype, *HER2* mutations were found more commonly in adenocarcinoma or adenosquamous cell carcinoma cases

Table 2. Patients recommended for HER2 alterations testing by guidelines in NSCLC

Guidelines	*HER2* mutation	*HER2* amplification
NCCN (V5.2021)[30]	(i) Testing for other genetic variants may also be done—such as NTRK gene fusions, *MET* amplification and *ErbB2* (also known as *HER2*) mutations—to identify these rare oncogenic driver variants for which effective therapy may be available, although there is less evidence to support testing. (ii) Broad molecular profiling is also recommended to identify rare driver mutations for which effective therapy may be available, such as *NTRK* gene fusions, high-level *MET* amplification, *ErbB2* mutations and *TMB*. Although clinicopathologic features—such as smoking status, ethnicity, and histology—are associated with specific genetic variants (e.g. *EGFR* mutations), these features should not be used to select patients for testing.	(i) For patients with an underlying *EGFR* sensitizing mutation who have been treated with EGFR TKI, minimum appropriate testing includes high sensitivity evaluation for p.T790M; when there is no evidence of p.T790M, testing for alternate mechanisms of resistance (*MET* amplification and *ErbB2* amplification) may be used to direct patients for additional therapies.
ASCO (2018)[31]	(i) *ErbB2* (*HER2*) molecular testing is not indicated as a routine stand-alone assay outside of the context of a clinical trial. It is appropriate to include *ErbB2* (*HER2*) mutation analysis as part of a larger testing panel carried out either initially or in case of negative routine *EGFR*, *ALK*, *BRAF* and *ROS1* testing.	No relevant recommendation

ALK, anaplastic lymphoma kinase; ASCO, American Society of Clinical Oncology; BRAF, v-Raf murine sarcoma viral oncogene homolog B1; EGFR, epidermal growth factor receptor; ErbB2/HER2, human epidermal growth factor receptor 2; HER2, human epidermal growth factor receptor 2; MET, mesenchymal-epithelial transition; NCCN, National Comprehensive Cancer Network; NSCLC, non-small-cell lung cancer; NTRK, neurotrophin tyrosine receptor kinase; ROS1, ROS proto-oncogene 1, receptor tyrosine kinase; TKI, tyrosine kinase inhibitor; TMB, tumor mutational burden.

($P = 0.012$), never smokers ($P < 0.0001$) and females ($P = 0.004$). Bu et al.[22] also found in NSCLC that HER2 insertions were proportionally more common in adenocarcinoma patients (91.4% versus 71.7%, $P = 0.01$), never smokers (97.1% versus 54.0%, $P < 0.01$) and females (91.4% versus 42.2%, $P < 0.01$) compared with the *HER2* insertion-negative group. A study carried out by Sholl et al.[23] showed that *HER2* mutations are significantly associated with never-smoking status ($P < 0.001$) and Asian origin ($P = 0.015$).

In addition, Offin et al.[24] reported that 47% of LC patients with *HER2* mutations have developed brain metastases at diagnosis (19%) or during treatment (28%), versus only 32% in LC patients with Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations; *HER2*-mutant patients were more likely to experience brain metastases during treatment in comparison with *KRAS*-mutant and *EGFR*-mutant diseases (*HER2*, 28%; *KRAS*, 8%; *EGFR*, 16%; *HER2* versus *KRAS*, $P < 0.001$; *HER2* versus *EGFR*, $P = 0.06$). Moreover, patients who experienced brain metastases had worse overall survival (OS) compared with those without brain metastasis. Yang et al.[4] reported that exon 20 YVMA insertion is notably associated with higher lifetime incidence of brain metastasis in advanced NSCLC ($P = 0.002$), with an estimated 12-month brain metastasis incidence of 40.2% compared with 3.6% in non-YVMA cases. These studies highlighted the importance of developing *HER2*-targeted agents with higher ability to penetrate the blood-brain barrier.[25] Moreover, lung adenocarcinomas with *HER2* mutations exhibit a more aggressive behavior on enhanced computed tomography compared with *KRAS*- and *EGFR*-mutant controls, and show a more frequent nodal metastatic spread compared with *KRAS*-mutant controls.[26] A case report described a lung adenocarcinoma patient with lymphangitic spread and psammoma bodies harboring an *HER2* exon 20 insertion mutation.[27] Differing from *HER2* mutations, *HER2* amplification and HER2 overexpression are not notably associated with distinct clinical pathological characteristics.[28] Another study by Lee et al.[29] compared patients with *HER2* amplification and *HER2* mutations: adenocarcinoma histology (100% versus 73.3%, respectively, $P = 0.021$), non-smoking status (63.6% versus 26.7%, $P = 0.027$) and presence of liver metastasis (31.8% versus 0%, $P = 0.025$) were significantly higher in patients with *HER2* mutations than those with *HER2* amplification. Interestingly, *EGFR* mutation (40% versus 0%, $P = 0.002$) was more common in patients with *HER2* amplification. HER2 overexpression, in accordance with *HER2* amplification, more frequently occurs in adenocarcinoma than in squamous cell carcinoma.[28] In lung adenocarcinoma, *HER2* expression was reported to notably correlate with papillary predominant histology, whereas *HER2* amplification correlated well with pleural invasion.[2]

Current recommendations for HER2 alteration testing

Although there is little evidence to support routine testing for various *HER2* alterations at the current moment, the National Comprehensive Cancer Network (NCCN) guideline does suggest that; in addition to testing *EGFR*, anaplastic lymphoma kinase (*ALK*), *RET,* receptor tyrosine kinase (*ROS1*), v-Raf murine sarcoma viral oncogene homolog B (*BRAF*) and *KRAS*, less common oncogenic driver gene mutations such as *HER2* mutations and amplification, among others, should be tested to guide effective treatment.[30] The American Society of Clinical Oncology (ASCO) guideline suggests that *HER2* molecular testing should not be routinely carried out independent of clinical trials; however, it does recommend *HER2* mutations to be tested as part of a larger testing panel carried out initially or in patients with negative test results for classic oncogenic genes, including *EGFR*, *ALK*, *BRAF* and *ROS1*[31] (Table 2). *HER2* amplification testing is recommended in clinical studies or in case of EGFR TKI resistance. The recommendations of these guidelines may principally be affected by the availability of targeted drugs for *HER2* alterations.

Besides, due to insufficient data for *HER2* expression in patients with NSCLC, *HER2* protein detection is not recommended for routine testing in current NCCN and ASCO guidelines. There are no recommendations from the European Society for Medical Oncology (ESMO) regarding the testing of *HER2* mutation, amplification and *HER2* expression.

We recommend that *HER2* mutation testing should be carried out upfront as part of a larger routine testing panel using next-generation sequencing (NGS), preferentially sequencing exon 20 of *HER2*. In patients with unresectable stage III and IV NSCLC who meet two or three of the following criteria, *HER2* mutation testing is recommended: (i) lung adenocarcinoma or adenosquamous carcinoma; (ii) never-smoking status; (iii) female.

Evidence suggesting companion diagnostics and treatment in NSCLC with *HER2* amplification and *HER2* expression is limited. Thus, *HER2* amplification and expression testing is not routinely recommended for all NSCLC patients. NGS and FISH for *HER2* amplification as well as IHC for *HER2* expression are recommended if tests are needed, especially for individuals in clinical studies and in case of EGFR TKI resistance, to explore the related resistance mechanisms.

CONSENSUS ON THERAPIES FOR NSCLC PATIENTS WITH *HER2* ALTERATIONS

Trastuzumab is a monoclonal antibody targeting *HER2*, which improves the outcome of *HER2*-positive BC.[32,33] Trastuzumab or another *HER2*/*HER3*-targeted drug, pertuzumab, however, demonstrated minimal clinical value with an ORR of only 13% in NSCLC patients with *HER2* amplification/overexpression.[20] The ORR was 29% in *HER2* exon 20-mutant NSCLC patients who received trastuzumab and pertuzumab in combination with docetaxel.[34] Currently, there is no FDA-approved targeted therapy for *HER2*-positive NSCLC. Chemotherapy shows unsatisfactory efficacy. It was reported that *HER2*-mutant NSCLC patients derive less benefit from pemetrexed-based chemotherapy (mPFS 5.1 months) than those with *ALK*/*ROS1* rearrangements (mPFS 9.2 months, $P = 0.004$).[35] Immune checkpoint inhibitors (ICIs) targeting the programmed cell death protein 1 and programmed death-ligand 1 (PD-L1) axis, including pembrolizumab and nivolumab, have demonstrated superiority over chemotherapy.[36-38] Recent research, however, showed that *HER2*-mutant subgroups do not derive similar benefit from these ICIs: the ORR in NSCLC patients with *HER2* alteration administered ICI monotherapy was 7%, which was much lower than those of patients with *KRAS* (26%), *BRAF* (24%), *ROS1* (17%), *MRT* (16%) and *EGFR* (12%) mutations.[39] Chen et al.[40] also reported an ORR of 0 (0/6) among *HER2*-mutant NSCLC patients treated with immunotherapy, and PD-L1 expression was significantly higher in patients with *EGFR* mutations than in those with *HER2* mutations (48.6% versus 19.0%, $P = 0.027$). These studies highlight the urgent clinical need to develop novel therapeutic strategies for NSCLC with various *HER2* alterations.

Recently, ADCs have shown promising therapeutic effects in early clinical studies (Table 3). Based on these trials, two ADCs (T-DM1 and T-DXd) were recommended for NSCLC with *HER2* mutations in the NCCN 2021 guidelines.[30] T-DXd is a novel ADC composed of an anti-*HER2* antibody, a cleavable tetrapeptide-based linker and a topoisomerase I inhibitor payload. In a phase II trial, *HER2*-mutant NSCLC patients administered T-DXd had a confirmed ORR of 72.7% (8/11) and an mPFS of 11.3 months.[41] DESTINY-Lung01 is an ongoing, multicenter, phase II study of T-DXd in patients with nonsquamous NSCLC overexpressing *HER2* or containing an *HER2*-activating mutation. In the DESTINY-Lung01 study, T-DXd demonstrated an ORR of 55% and an mPFS of 8.2 months in the *HER2*-mutant NSCLC cohort.[7] T-DM1, an ADC incorporating the *HER2*-targeted monoclonal antibody trastuzumab with the cytotoxic microtubule inhibitor DM1, was shown to be effective and tolerable in 18 patients with *HER2*-mutant NSCLC in a clinical study conducted in the USA, with an ORR of 44% and an mPFS of 5 months.[42] A phase II study of T-DM1 monotherapy in relapsed *HER2*-positive NSCLC was terminated early, however, due to limited efficacy. This trial showed that among 15 *HER2*-positive (IHC 2+/3+ and FISH-positive, or exon 20 mutation) patients, only 1 with *HER2* mutation achieved a partial response.[43]

The therapeutic values of *HER2* TKIs, including afatinib, dacomitinib, neratinib, poziotinib and pyrotinib are summarized in Table 3. Afatinib was reported to have antitumor activity in pretreated *HER2*-mutant NSCLC patients, with an ORR of 19% (3/16) and a disease control rate (DCR) of 69% (11/16), especially in the subgroup harboring the p.A775_G776insYVMA insertion in exon 20, which had an ORR of 33% (2/6) and a DCR of 100% (6/6).[44] A retrospective multi-centered study in Chinese patients, however, reported an opposite outcome that afatinib yielded no response in the YVMA subgroup.[45] Afatinib was recommended by NCCN guidelines (2018 update, version 1.0) as single-agent therapy for *HER2*-mutant NSCLC; however, this recommendation was subsequently omitted in NCCN guidelines version 3, 2018, due to poor response.[46] In a single-arm phase II trial, afatinib therapy resulted in a lower DCR than expected in NSCLC harboring *HER2* exon 20 mutations.[47] A retrospective, nationwide study showed that chemotherapy might bring more benefit than afatinib in *HER2*-mutant advanced LC cases, particularly in those with A775_G776insYVMA.[48] A recently published study may explain this result by suggesting that YVMA insertion in the HER2 kinase domain generated a more rigid conformation, which led to less potent inhibition by TKI monotherapy and the greater need of adding chemotherapy.[49] Another TKI targeting *HER2*, pyrotinib, was reported to have superior antitumor activity over afatinib and T-DM1 in both $HER2^{YVMA}$ insertion patient-derived organoid and xenograft models, with significant inhibition of pHER2 and downstream pERK and pAKT. In addition, pyrotinib also demonstrated promising clinical efficacy in 15 *HER2*-mutant NSCLC

Table 3. Efficacy of targeted drugs in NSCLC with HER2 alterations

Class	Drugs	Targets	Study type	Patients	Total number	Efficacy in NSCLC with HER2 alterations			
						ORR, %	Median PFS, months	Median OS, months	References
Humanized monoclonal antibody	Trastuzumab + pertuzumab	HER2	Phase IIa	HER2, EGFR, BRAF or Hedgehog pathway-altered advanced refractory solid tumors	251 (Total) 30 (HER2-altered NSCLC treated with trastuzumab + pertuzumab)	13% (2/16): *HER2*-amplified or HER2-overexpressed NSCLC 21% (3/14): *HER2*-mutant NSCLC	NA	NA	(Hainsworth et al., 2018)[20]
			Phase II	*HER2* exon 20-mutant advanced NSCLC	45	29% (13/44)	6.8 (4.0-8.5)	NA	(Mazieres et al., 2021)[34]
ADC	T-DM1	HER2	Phase II	*HER2*-mutant advanced NSCLC	18 (Total) 11 (*HER2* exon 20 mutations)	44% (8/18): Total 54.5% (6/11): *HER2* exon 20 mutations	5.0 (3.0-9.0): total	NA	(Li et al., 2018)[42]
			Phase II	Pretreated HER2-positive (IHC/FISH/mutant-positive) NSCLC	15 (Total) 7 (*HER2* exon 20 mutations)	6.7% (1/15): Total 14.3% (1/7): *HER2* exon 20 mutations	2.0 (1.4-4.0): total	10.9 (4.4-12.0): total	(Hotta et al., 2018)[43]
			Phase II	HER2-overexpressed NSCLC	49	0% (0): IHC (2+) 20% (4/20): IHC (3+)	2.6 (1.4-2.8): IHC (2+) 2.7 (1.4-8.3): IHC (3+)	12.2 (3.8-23.3): IHC (2+) 15.3 (4.1-NE): IHC (3+)	(Peters et al., 2019)[96]
			Phase II	*ErbB2*- and/or mutant lung cancers	49 (Total) 11 (*ErbB2* amplification) 28 (*ErbB2* mutation) 10 (concurrently *ErbB2* mutation and amplification)	51% (25/49): Total 55% (6/11): *ErbB2* amplification 50% (14/28): *ErbB2* mutations 50% (5/10): concurrently *ErbB2* mutation and amplification	5.0 (3.5-5.9): total	NA	(Li et al., 2020)[55]
	T-DXd		Phase I	Pretreated, HER2-overexpressed (IHC ≥1+), non-breast/non-gastric or *HER2*-mutant solid tumors	60 (Total) 18 (HER2-overexpressed or -mutant NSCLC) 11 (*HER2*-mutant NSCLC)	55.6% (10/18): HER2-overexpressed or -mutant NSCLC 72.7% (8/11): *HER2* -mutant NSCLC	11.3 (7.2-14.3): HER2-overexpressed or -mutant NSCLC 11.3 (8.1—14.3): *HER2*-mutant NSCLC	NA	(Tsurutani et al., 2020)[41]
			Phase II	HER2-overexpressed or *HER2*-mutant metastatic NSCLC	49 ((HER2-overexpressed) 42 (*HER2* mutation)	24.5% (12/49): HER2-overexpressed 61.9% (26/42): *HER2* mutation	5.4 (2.8-7.0): HER2-overexpressed 14.0 (6.4-14.0): *HER2* mutation	NA	(Nakagawa et al., 2021)[54]; (Smit et al., 2021)[97]
TKI	Afatinib	EGFR, HER2 and HER4	Phase I	*HER2*-mutant positive NSCLC	80	0	2.76 (NA)	10.02 (NA)	(Fan et al., 2020)[98]
			Phase II	Pretreated *HER2* exon 20-mutant advanced NSCLC	13	7.7% (1/13)	15.9 Weeks (6.0-35.4)	56 Weeks (16.3-NE)	(Dziadziuszko et al., 2019)[47]
			Retrospective study	*HER2*-mutant advanced NSCLC	32	15.6% (5/32)	3.2 (2.0-4.5)	NA	(Fang et al., 2020)[45]
			Retrospective study	HER2-altered NSCLC	66 (Total) 54 (*HER2* mutations) 12 (*HER2* amplification)	24% (16/66): Total 22% (12/54): *HER2* mutations 33% (4/12): *HER2* amplification	3.3 (2.2-4.4): total 3.4 (1.4-4.7): *HER2* mutations 3.3 (2.6-4.0): *HER2* amplification	13.9 (11.4-16.5): total 14.6 (11.6-17.6): *HER2* mutations 13.4 (0-27.6): *HER2* amplification	(Song et al., 2021)[56]

Continued

Table 3. Continued

Class	Drugs	Targets	Study type	Patients	Total number	Efficacy in NSCLC with HER2 alterations			
						ORR, %	Median PFS, months	Median OS, months	References
	Dacomitinib	HER2, EGFR and HER4	Phase II	*HER2*-mutant or amplified advanced NSCLC	30 (Total) 26 (*HER2* mutations)	11.5% (3/26): *HER2* mutations 0 (0): *HER2* amplification	3 (2-4): *HER2* mutations NA (1-5): *HER2* amplification	9 (7-21): *HER2* mutations NA (5-22): *HER2* amplification	(Kris et al., 2015)[99]
	Neratinib	HER2, EGFR and HER4	Phase II	*HER2*- and *HER3*-mutant cancers	141 (Total) 26 (*HER2*-mutant lung cancer)	NA	5.5 (NA): *HER2* mutant lung cancer	NA	(Hyman et al., 2018)[100]
	Poziotinib	HER2, EGFR and HER4	Phase II	NSCLC with *EGFR* or *HER2* exon 20 mutations	205 (Total) 90 (*HER2* mutations)	27.8% (NA): *HER2* mutations	NA	NA	(Cornelissen et al., 2021)[52]
			Phase II	NSCLC patients with *EGFR* or *HER2* exon 20 mutations	30 (Total) 8 (*HER2* mutations)	50% (4/8): *HER2* mutations	NA	NA	(Prelaj et al., 2021)[53]
	Pyrotinib	HER2, EGFR, and HER4	Phase II	Pretreated *HER2* exon 20-mutated advanced NSCLC	15	53.3% (8/15)	6.4 (1.60-11.20)	12.9 (2.05-23.75)	(Wang et al., 2019)[50]
			Phase II	Pretreated *HER2*-mutant advanced lung adenocarcinoma	60	30% (18/60)	6.9 (5.5-8.3)	14.4 (12.3-21.3)	(Zhou et al., 2020)[51]
			Phase II[a]	Pretreated NSCLC patients with diverse HER2 alterations	33	45.5% (15/33)	6.8 (5.3-9.77)	NE (10.23-NE)	(Yang et al, 2021)[101]

ADC, antibody-drug conjugates; BRAF, v-Raf murine sarcoma viral oncogene homolog B1; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; HER3, human epidermal growth factor receptor 3; HER4, human epidermal growth factor receptor 4; IHC, immunohistochemistry; NE, not evaluable/missing; NA, not available; NSCLC, non-small-cell lung cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; T-DM1, trastuzumab emtansine; T-DXd, trastuzumab deruxtecan; TKI, tyrosine kinase inhibitor.

[a] The treatment regimen in this study was pyrotinib combined with apatinib.

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patients, with an ORR of 53.3% and an mPFS of 6.4 months.[50] In a subsequent multicenter phase II clinical study enrolling 60 *HER2*-mutant lung adenocarcinoma patients previously treated with platinum-based chemotherapy, pyrotinib resulted in an ORR of 30%, an mPFS of 6.9 months and a median OS of 14.4 months.[51] Another study also assessed the clinical effect of poziotinib in previously treated NSCLC patients harboring *EGFR* and *HER2* exon 20 mutations, with ORRs of 14.8% and 27.8%, respectively. In addition, ORRs were 30% and 39%, respectively, in *HER2*-mutant LC patients who received two or three lines of therapy.[52] Furthermore, poziotinib demonstrated benefits in metastatic NSCLC with *EGFR*/*HER2* exon 20 insertion mutation, with an ORR of 30% (*EGFR*/*HER2*: 23%/50%) and a DCR of 80%.[53]

A few trials have assessed targeted agents in NSCLC patients with *HER2* amplification and/or overexpression (Table 3). The DESTINY-Lung01 study also enrolled patients with *HER2*-overexpressing metastatic NSCLC, with an ORR of 24.5% and an mPFS of 5.4 months.[54] In a study where *HER2*-mutant and/or -amplified NSCLC patients were treated with T-DM1,[55] the ORR of *HER2*-amplified, and concurrently *HER2*-mutant and -amplified patients were 55% and 50%, respectively. A phase II study of T-DM1 in Japan, however, showed no definitive benefit in *HER2*-positive NSCLC patients.[43] A multicenter retrospective study included metastatic NSCLC patients harboring *HER2* alterations administered afatinib, revealing an ORR of 33%, an mPFS of 3.3 months and a median OS of 13.4 months in *HER2* amplification.[56] These results indicate that effective targeted drugs for NSCLC with *HER2* amplification and/or overexpression need to be further investigated.

Several ongoing studies in *HER2*-altered NSCLC patients are listed on ClinicalTrials.gov (https://clinicaltrials.gov/). These include PYRAMID-1 (NCT04447118), a phase III randomized clinical trial comparing pyrotinib and docetaxel in patients with advanced nonsquamous NSCLC harboring *HER2* exon 20 mutation with failed platinum-based chemotherapy; DESTINY-Lung02 (NCT04644237), a phase II cohort study of T-DXd in *HER2*-mutated metastatic NSCLC patients; DESTINY-Lung03 (NCT04686305), a phase Ib study investigating the safety of T-DXd in combination with immunotherapy and chemotherapy in patients with *HER2*-positive advanced and metastatic NSCLC; a phase IIb study (NCT04311034) of RC48 in patients with *HER2*-overexpressing or *HER2*-mutant NSCLC; and a phase I/II study (NCT04818333) of SHR-A1811 (*HER2* ADC) in *HER2*-altered NSCLC patients. In summary, targeted drugs, such as *HER2*-targeted ADCs and pyrotinib, are being actively examined with the expectation that they might be new treatment options for NSCLC with *HER2* mutations and other *HER2* alterations.

RECOMMENDATIONS FOR *HER2* ALTERATIONS TESTING IN NSCLC

Sample collection

Tumor tissues, cytologic specimens and circulating tumor DNA (ctDNA) could be used for *HER2* testing. Tumor tissues are preferred and should contain a substantial part of tumor cells without obvious necrosis, mucus and inflammatory changes. Alternatively, cytologic specimens and ctDNA can be used.[30,31] It is well established that ctDNA, a tumor-specific DNA fragment released into plasma from apoptotic and necrotic tumor cells, can be used for mutations detection in NSCLC.[57-59] Mack et al.[60] demonstrated the ability to detect *HER2* mutations in ctDNA from NSCLC cases, with 126 mutations identified in a series of >8000 plasma samples analyzed on a commercially available NGS platform. In this series, small in-frame insertions in exon 20 represented >60% of cases, and *HER2* mutations were significantly mutually exclusive with other known NSCLC driver genes. Considering that the false-negative rate of ctDNA testing is as high as 30%, tissue collection for re-testing is recommended in case the initial peripheral blood ctDNA testing fails to identify *HER2* mutations.[30]

Sample processing and storing for HER2 alteration testing

Time from tissue acquisition to fixation (within 1 h) or storage in liquid nitrogen (within 10 min) should be as short as possible, and tissue specimens should be sliced at 5- to 10-mm intervals and fixed in sufficient volume of 10% neutral buffered formalin for 6-72 h.[61,62] Unstained sections should not be left at room temperature for >6 weeks to prevent antigen loss.[61] When ctDNA in plasma extracted from peripheral blood is collected for testing, disposable closed EDTA anticoagulant vacuum blood collection tubes should be used for sampling. Alternatively, Streck tubes or other cell-free DNA collection tubes can be used, granting additional storage time at room temperature before processing. Minimally 6-10 ml of whole blood should be collected; the plasma should be separated using a double-spin technique, and ctDNA should be extracted within 6 h. Finally, ctDNA should be stored at −80°C, and repeated thawing should be avoided.[63] Additional considerations for ctDNA collection and extraction from plasma are provided in Rolfo et al.[63]

A sufficient proportion of tumor cells in samples is the key to determining the reliability of the test results. A study including 665 lung adenocarcinoma specimens (558 TKI-naive and 107 TKI-recurrent samples) explored the effect of tumor cellularity on NGS test results. It was found that biopsied samples with <20% tumor cellularity are associated with lower frequency of *HER2* mutations compared with those with ≥20%.[64] Literature suggested minimal tumor cell content should exceed twice the limit of detection of the testing method used.[65] Accordingly, the optimal tumor cell content in tissue samples for NGS is 40%, and the minimum is 10%-20%.[66] In addition to the number of tumor cells, intratumor heterogeneity should not be ignored during genetic testing, as it may result in inaccurate findings, especially false-negatives.[67] It was shown that intratumor heterogeneity exists in *HER2*-mutant lung adenocarcinomas, and the heterogeneity score of *HER2* is significantly higher in metastatic tumors compared with primary tumors.

Therefore, it is crucial to carry out preassessment before sample processing to achieve optimal test quality.[64]

Techniques and platforms for detecting HER2 mutations

The methods used to assess *HER2* mutations mainly include Sanger sequencing, NGS, amplification refractory mutation system-PCR (ARMS-PCR) and droplet digital PCR (ddPCR). These methods have different sensitivities, specificities and sample requirements, and vary in the types of genetic alterations tested, difficulty of operation and speed of testing. It is recommended to carefully select the testing method based on local laboratory conditions, sample type, sample size and clinical needs.

Sanger sequencing can read a given DNA sequence directly and identify new mutation sites; it has high requirements regarding the content and proportion of tumor cells in the sample, and is not suitable for small biopsies or cytological specimens.[68,69] ARMS-PCR shows high sensitivity and specificity, and its operation is simple; however, it cannot identify new and unknown mutations. When different mutation sites need to be tested, the required DNA amount for ARMS-PCR increases, and the probability of non-specific binding increases correspondingly.[70] The ddPCR method also has excellent sensitivity and specificity, but no advantage in processing samples with high DNA concentrations.[71] NGS can sequence millions or even billions of DNA molecules simultaneously; in particular, it requires less DNA for testing and shows high sensitivity.[59,72,73] Therefore, NGS is recommended for *HER2* mutation testing. An ideal NGS testing platform should be able to identify all types of variations in *HER2* related to clinical treatment, including exon-20 YVMA insertions, non-YVMA insertions, missense point mutations, copy number variation and amplification, etc., with low requirement of required DNA amount, high speed and high repeatability.

Techniques for detecting HER2 amplification

Gene amplification refers to an increase in copy number of a specific chromosomal location in comparison with the remainder.[74] *HER2* copy number elevation can occur through focal amplification or a balanced copying of chromosome 17 where *HER2* is located (defined as polysomy).[75] A study conducted by Han et al.[76] showed that focal amplification of *HER2* may predict early relapse after adjuvant chemotherapy in patients with lung adenocarcinoma. In NSCLC tumors, however, increased copy number of *HER2* is largely due to the polysomy of chromosome 17.[77,78] Although several studies showed that high *HER2* gene copy number is associated with reduced survival in LC, the prognostic and therapeutic implications of polysomy in LC need to be examined in prospective clinical trials.[77,79]

Various techniques can be used for detecting *HER2* copy number changes. These include NGS, real-time quantitative (qRT)-PCR and FISH. In current clinical practice, NGS is a commonly used method for *HER2* amplification in NSCLC. There is currently no uniform standard for defining amplification across NGS platforms. The advantage of NGS-based testing for copy number changes lies in its ability to assess variants across hundreds of genes simultaneously and to identify focal gene amplifications from numerous chromosomal gains, with a high level of resolution. Although qRT-PCR has also been used to detect *HER2* amplification, this method has no obvious advantage over NGS; besides, the cut-off points used to define *HER2* amplification vary, and no clear definition criterion has been proposed.

FISH, a technique leveraging fluorophore-coupled DNA fragments to tag and detect target genomic regions, is recommended for *HER2* amplification testing in NSCLC clinical studies for more evidence. As a dual-probe technique, FISH allows the determination of copy number changes for both the *HER2* gene and the centromere of chromosome 17 (CEP17).[80] *HER2* copy number gain can be defined by calculating the gene copy number or the ratio of *HER2* to CEP7. In comparison with the mean *HER2* copy number, the *HER2*/CEP17 ratio is sometimes considered a better reflection of *HER2* amplification status, as the former may be influenced by multiple factors, including abnormal chromosome copy number (aneusomy), mitotic index of the tumor, section thickness and nuclear truncation effects.[81] The interpretation of FISH test results in NSCLC could refer to BC as follows. (i) *HER2* to CEP17 ratio $\geq$2.0, positive result indicating *HER2* amplification. (ii) *HER2* to CEP17 ratio $<$2.0: *HER2* copy number $\geq$6.0, positive result indicating *HER2* amplification; *HER2* copy number $<$4.0, negative result indicating no *HER2* amplification; *HER2* copy number $\geq$4.0 but $<$6.0, uncertain result, with not determinable *HER2* status. (iii) If numerous *HER2* signals are connected into clusters, there is no need to calculate as this is clearly indicative of *HER2* amplification.[82] In clinical studies, FISH is recommended for *HER2* amplification testing in NSCLC.

Techniques for detecting HER2 overexpression

IHC is recommended as a standard method for the detection of *HER2* expression in solid tumors such as BC, gastric cancer, intestinal cancer and NSCLC. *HER2* IHC can be assessed using two different methods, including the H-scoring system and ASCO/College of American Pathologist (CAP) BC guidelines. H-scoring assessment is determined by multiplying the intensity of staining (0-3) by the percentage of positive cells (0%-100%), with a possible range from 0 to 300. A score $>$200 is generally considered to indicate overexpression, but the cut-offs of the H-scoring system vary in different studies.[2,83] More research is needed to achieve an applicable standard in NSCLC.

In the ASCO/CAP guidelines for BC, the final score is 0, 1+, 2+ or 3+ based on membranous staining, among which scores of 0/1+ and 3+ are considered to be negative and positive for overexpression, respectively; a score of 2+ is considered equivocal and needs to be confirmed by additional *in situ* hybridization testing.[61] Since *HER2* expression is not routinely tested in clinical practice for NSCLC, detection of *HER2* overexpression in NSCLC in general follows the diagnostic criteria for BC.[82,84,85] Intriguingly, there is no obvious correlation between *HER2* amplification

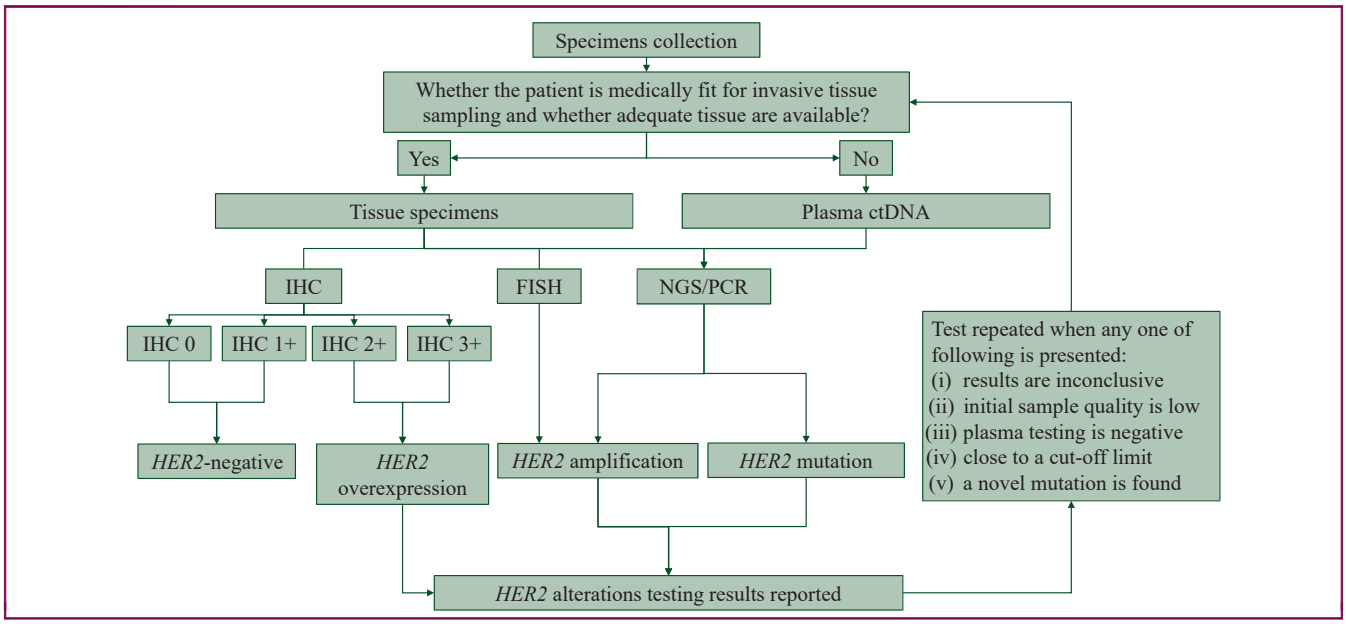

Figure 1. Recommended algorithm for testing HER2 alterations in non-small-cell lung cancer.
The definitions of HER2 expression and amplification in this consensus were determined based on available clinical studies; further verification is recommended when *HER2* amplification is detected by NGS or PCR.
ctDNA, circulating tumor DNA; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; NGS, next-generation sequencing.

and overexpression in NSCLC, which is in sharp contrast with BC. A study was designed to elucidate the concordance between *HER2* IHC and ISH in NSCLC; the results showed that the concordance rate of the *HER2* IHC (2+) subgroup was 0.091, which was much lower than 0.975 found in the *HER2* IHC (0/1+) subgroup.[86] Another study also reported the poor concordance between IHC and silver-enhanced *in situ* hybridization. In addition, the sensitivity and specificity of IHC for detecting amplification were 23.9% and 94.9% at a cut-off of ≥2+, respectively.[2] These findings indicate that detection of *HER2* protein expression in NSCLC is different from that in BC, as there are much less IHC (2+) cases in FISH-positive LC patients, and IHC 2+ to detect *HER2* amplification has poor sensitivity. Therefore, we recommend FISH confirmation is not required for NSCLC patients with *HER2* IHC 2+. Companion diagnostic criteria of *HER2* expression in NSCLC is suggested as follows: (i) 0, *HER2* expression negative; (ii) 1+, currently considered to be negative. With the increased application of ADC therapy and update of research evidence,[87] however, it needs to be confirmed whether 1 + should be considered to be *HER2* expression negative or *HER2*-low expression; (iii) 2+, 3+: *HER2* expression positive.

Testing reports and laboratory requirements

Reports should contain the following information: sample source, size and quality, methodology used and assay sensitivity. Critically, for mutation reporting, the specific amino acid substitutions must be reported, as different point mutations and insertions will likely prove to be differentially responsive to the various targeted agents currently available or in development. Samples used for DNA extraction should also indicate the content of tumor cells.[88] In an *HER2* amplification report, both the *HER2*/CEP17 ratio and the copy number of *HER2* should be included.

When testing results near the cut-off limits resulting in difficulty to determine, an alternative method for re-testing should be recommended, or the relevant information should be provided in the testing report to notify the clinician. If a novel (unreported) mutation is observed, testing should be repeated to avoid false-positives. In case of negative peripheral blood tests, we recommend the clinician to closely monitor the patient's disease status, reassess the feasibility of tissue biopsy, and re-test *HER2* with the tissue or other samples as appropriate. It should be cautioned that the presence of molecular alterations cannot be ruled out when the quality/quantity of specimens used for testing are low and/or when the results are negative.

Laboratories carrying out *HER2* alteration testing should meet national and international quality standards and be accredited by relevant bodies [e.g. CAP and Clinical Laboratory Improvement Amendments (CLIA)].[30,89] The laboratories must participate in regular quality control programs such as Pathology Quality Control Center (PQCC) and European Molecular Genetics Quality Network (EMQN) and other inter-laboratory quality assessment programs on an annual basis. When testing results are inconsistent (with a low confidence level) or otherwise unexpected, the laboratory should ensure that there are available alternative methods or samples to overcome these challenges.[31] The testing personnel should have relevant educational background and corresponding work experience, with professional training and relevant qualification certificates, and

Table 4. Key points of the consensus on the testing of HER2 alterations in NSCLC

1. Epidemiology of HER2 alterations in NSCLC
(i) The incidence of *HER2* mutation in Asian populations seems to be numerically higher than that of the European and US populations (1.4%-6.7% versus 1%-3%). *HER2*-ex20ins is the most common mutation (48%);
(ii) The incidence rates of *HER2* amplification and HER2 expression vary in different studies, which needs further investigation.

2. Clinicopathologic features of HER2 alterations in NSCLC
(i) NSCLC with *HER2* mutations are more likely to occur in adenocarcinoma or adenosquamous carcinoma, never smokers and females;
(ii) No distinct characteristics are observed in NSCLC patients with *HER2* amplification or HER2 expression.

3. Current recommendations for HER2 alterations testing
(i) *HER2* mutation testing should be carried out as part of an initial larger testing panel applying next-generation sequencing, and exon 20 of *HER2* mutations should be preferentially included;
(ii) In patients with unresectable stage III and stage IV NSCLC meeting two or three of the following criteria, *HER2* mutation testing is recommended whenever possible: (a) lung adenocarcinoma or adenosquamous carcinoma; (b) no or mild smoking history; and (c) female sex.
(iii) *HER2* amplification is recommended when resistance to EGFR TKI develops. In addition, *HER2* amplification and expression are recommended for NSCLC in clinical trials.

4. Therapies for NSCLC patients with HER2 alterations
(i) Monoclonal anti-HER2 antibodies, chemotherapy with or without ICIs targeting PD-1/PD-L1 have limited efficacy in NSCLC with HER2 alterations;
(ii) ADCs (e.g. T-DXd, T-DM1) and TKIs (e.g. pyrotinib) are expected to be new treatment options for NSCLC with HER2 alterations. Targeted therapies for NSCLC with HER2 overexpression need to be further investigated.

5. Sample collection for the testing of HER2 alterations
(i) Tumor tissue is preferred for HER2 testing whenever it is available;
(ii) In case of unavailable or too small tissue sample, other specimens, such as ctDNA, should be used.

6. Techniques and platforms for detecting *HER2* mutations
(i) Sanger sequencing, ARMS-PCR, ddPCR and NGS can all be used for *HER2* mutation testing. NGS is preferred, and an ideal NGS testing platform should be able to identify all types of *HER2* exon 20 mutations related to clinical treatment, including exon 20 YVMA insertions, non-YVMA insertions and missense mutations.

7. Techniques and platforms for detecting *HER2* amplification
(i) FISH is recommended for the testing of *HER2* amplification in NSCLC-related clinical studies. In current clinical practice, NGS is a commonly used method for detecting *HER2* amplification in NSCLC.
(ii) *HER2* amplification criteria by FISH
- ◇ *HER2* to CEP17 ratio $\geq$2.0: *HER2* amplification positive;
- ◇ *HER2* to CEP17 ratio $<$2.0:
  - *HER2* copy number $\geq$6.0: *HER2* amplification positive;
  - *HER2* copy number $<$4.0: *HER2* amplification negative;
  - *HER2* copy number $\geq$4.0 but $<$6.0: amplification status cannot be determined;
- ◇ Numerous *HER2* signals connected into clusters: no need to be calculated, i.e. *HER2* amplification positive.

8. Techniques and platforms for detecting HER2 expression
(i) Although HER2 expression is not frequently tested in clinical practice in NSCLC, IHC is recommended as the standard method for the detection of HER2 expression;
(ii) HER2 expression criteria by IHC
- ◇ 0: HER2 expression negative;
- ◇ 1+: needs to be confirmed by further studies whether 1+ should be considered to be negative or HER2-low expression;
- ◇ 2+, 3+: HER2 expression positive.
- ◇ Due to the poor concordance between FISH and IHC in NSCLC, FISH confirmation is not required for NSCLC patients with IHC 2+/3+ to define positive HER2 expression.

9. Future directions and optimization strategies for detecting HER2 alterations
(i) Further refinement of the testing procedure and companion diagnostics for *HER2* mutation, amplification and HER2 expression are needed;
(ii) Clinical trials in NSCLC patients with HER2 alterations should be encouraged to provide high-quality evidence so that relevant detection methods can be further optimized;
(iii) Exploration of HER2 TKI resistance mechanisms in NSCLC with HER2 alterations is warranted.

ADC, antibody-drug conjugate; ARMS-PCR, amplification refractory mutations system-PCR; CEP17, chromosome 17 centromere; ctDNA, circulating tumor DNA; ddPCR, droplet digital PCR; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; ICIs, immune checkpoint inhibitors; IHC, immunohistochemistry; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; T-DM1, trastuzumab emtansine; T-DXd, trastuzumab deruxtecan; TKI, tyrosine kinase inhibitor.

operate in strict accordance with the standard operating procedures.

CONCLUSION

The testing procedure and companion diagnostics of *HER2* alterations need to be standardized worldwide. Here, we reached a consensus and recommended an algorithm for testing HER2 alterations in non-small-cell lung cancer (Table 4, Figure 1). More translational studies are warranted for establishing the criteria for *HER2* companion diagnostics of *HER2* amplification and *HER2* expression, and confirming the relationships among *HER2* mutation, amplification and protein expression in NSCLC. The definitions of *HER2* expression and amplification in this consensus were determined based on available clinical studies. Further verification is recommended when *HER2* amplification was detected by NGS or PCR.

Several targeted drugs have shown promising efficacy in *HER2*-altered NSCLC patients. The optimal management of *HER2*-altered NSCLC, however, requires further high-quality trials (e.g. randomized phase III studies) and the exploration of new treatment strategies such as ADC combined with TKI, ICIs or chemotherapy for *HER2* alterations, as well as TKI or ADC for *HER2* amplification, etc. In addition, understanding the mechanisms underlying TKI or ADC resistance in NSCLC with *HER2* alterations is warranted. Therefore, more attention needs to be paid to *HER2*-altered NSCLC, which would also help refine the companion

diagnosis and treatment of *HER2* alterations in other solid tumors such as gastric cancer, intestinal cancer and urothelial carcinoma.

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