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REVIEW

Targeting HER2 in non-small-cell lung cancer (NSCLC): a glimpse of hope? An updated review on therapeutic strategies in NSCLC harbouring HER2 alterations

M. Riudavets[1], I. Sullivan[2], P. Abdayem[1] & D. Planchard[1*]

[1]Department of Cancer Medicine, Gustave Roussy Cancer Campus, Villejuif, France; [2]Medical Oncology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

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Non-small-cell lung cancer (NSCLC) harbouring HER2 alterations is now considered a distinct molecular subtype. The activation of HER2 in NSCLC occurs via three mechanisms, i.e. gene mutation (1%-4% of cases), gene amplification (2%-5%) and protein overexpression (2%-30%), with different prognostic and predictive outcomes. So far, non-selective tyrosine kinase inhibitors (TKIs) have shown a minor benefit in *HER2*-mutant NSCLC patients with objective response rates (ORRs) ranging from 0% to 19%. Trastuzumab-based chemotherapy was not found to be superior to chemotherapy alone [median progression-free survival (PFS) 6.1 *versus* 7 months, respectively] and dual HER2 antibody blockade with trastuzumab and pertuzumab had limited efficacy (ORR 13%-21%). In contrast, novel more selective HER2 TKIs such as poziotinib and pyrotinib have shown a promising activity in *HER2*-mutant pre-treated NSCLC patients, with response rates up to 38% and 44%, respectively. The most encouraging data come from phase II studies that evaluated the antibody—drug conjugates (ADCs) ado-trastuzumab—emtansine and trastuzumab—deruxtecan in patients with *HER2*-mutant NSCLC, with response rates of 50% and 62%, respectively. These agents are bringing hope to the management of HER2-altered NSCLC. Moreover, a paradigm shift from monotherapies towards combinations of agents with distinct mechanisms of action, such as ADCs with irreversible TKIs or immune checkpoint inhibitors, is already taking place and will change the therapeutic landscape of HER2-driven NSCLC. This paper provides a practical, concise and updated review on the therapeutic strategies in NSCLC with HER2 molecular alterations.

Key words: non-small-cell lung cancer, *HER2* mutation, *HER2* amplification, HER2 overexpression, targeted therapies

INTRODUCTION: HER2 IN LUNG CANCER

Lung cancer is the leading cause of cancer-related mortality worldwide. Non-small-cell lung cancer (NSCLC), the main histologic subtype accounting for 85% of lung cancer cases, is a heterogeneous disease driven by a wide spectrum of molecular alterations.[1,2] Targeted therapies directed against specific molecular aberrations, such as epidermal growth factor receptor (*EGFR*) and B-RAF proto-oncogene serine/threonine kinase (*BRAF*) mutations, as well as anaplastic lymphoma kinase (*ALK*) and ROS proto-oncogene 1 receptor tyrosine kinase (*ROS1*) rearrangements, have indisputably improved both the prognosis and the quality of life of lung cancer patients, and are now a standard of care in oncogene-driven NSCLC.[2]

The human epidermal growth factor 2 receptor (*HER2*) gene, also known as *ErbB2*, is a known proto-oncogene that is located on the long arm of chromosome 17 (17q21). While *ErbB2* refers to the gene across both human and rodent species, *HER2* is used in reference to the human gene and the gene product. The term *Neu* alludes to its rodent counterparts, since the first evidence of *HER2*'s role in cancer came from the connection to its rat ortholog, *Neu*, a mutated gene that was identified in carcinogen-induced neuroblastoma.[3-5] The HER2 protein product is a member of the HER/ErbB family of tyrosine kinases receptors. It consists of an extracellular region, a transmembrane domain and a tyrosine kinase domain with a C-terminal regulatory region.[3,4] HER2 does not have a known soluble ligand; downstream signalling is triggered by dimerization with other ligand-bound HER family members. HER2 is also less prone to internalization and degradation and can remain activated for a longer time on the cell membrane.[3,4]

*Correspondence to: Dr David Planchard, Department of Cancer Medicine, Gustave Roussy Cancer Campus, 114 Edouard Vaillant Street, F-94800 Villejuif, France. Tel: +33-01-42-11-51-48; Fax: 01-42-11-52-19

E-mail: David.PLANCHARD@gustaveroussy.fr (D. Planchard).

The common consequence of all the alterations in the *HER2* gene/protein is the receptor's hyperactivation following increased homo- or heterodimerization and autophosphorylation, which triggers multiple signalling pathways resulting in uncontrolled cell proliferation, such as mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT), protein kinase C (PKC) and signal transducers and activators of transcription (STAT).[3,4]

Three HER2 activating mechanisms have been described in NSCLC: gene mutation (1%-4% of cases), gene amplification (2%-5%) and protein overexpression (2%-30%).[6-8] Since *HER2* mutations have not been strictly associated with *HER2* amplification and overexpression, thus suggesting distinct mechanisms of origin and resulting in different clinical characteristics, different prognostic and predictive outcomes, *HER2*-mutant, *HER2*-amplified and HER2-overexpressing NSCLC patients should be considered as three distinct HER2-altered subgroups.[9,10]

HER2 mutations and amplifications have been associated with female sex, Asian ethnicity, non-smoking status as well as moderate to poorly differentiated adenocarcinoma histology. Pleural invasion is commonly seen in *HER2*-amplified and HER2-overexpressing NSCLC while central nervous system (CNS) involvement has been reported in up to 47% of patients with *HER2*-mutant NSCLC.[11-13]

While HER2 overexpression has been found in different studies to be associated with poor outcomes in NSCLC, the prognostic value of *HER2* mutation and amplification remains unclear.[14,15]

HER2 *mutations*

Exon 20 insertions affecting the kinase domain are the most frequent *HER2* mutations (96%).[11] As a group, they resemble *EGFR* exon 20 activating mutations (non-*T790M* mutations), which have been associated with primary resistance to both first- and second-generation tyrosine kinase inhibitors (TKIs).[16] In both cases, insertions are in-frame, ranging from 3 to 12 base-pairs (bp) and are all nested in the most proximal region of the exon. Compared to *EGFR* insertions, *HER2* insertions are less heterogeneous, with over 83% of the cases consisting of an insertion of 12 bp leading to the duplication of amino acids YVMA at codon 775, thus known as the A775_G776insYVMA insertion/duplication.[11,12] Among the genomic changes induced by the YVMA mutation, the 2326-2337 (TACGTGATGGCT) has never been described in Asian patients, while the 3-bp 2327-2329 insertion/duplication seems to be restricted to this subgroup.[11,17,18]

HER2 exon 20 mutations also include point mutations, such as L755S and G776C (8%-10% of all the identified *Her2* mutations).[11] Recently, a few less common mutations affecting the transmembrane and the juxtamembrane domains (G660D, R678Q, E693K and Q709L) have been reported.[19,20]

HER2 mutations and other oncogenic drivers, such as *EGFR, KRAS, NRAS, ALK, PI3KCA* and *BRAF*, have previously been shown to be mutually exclusive.[11,12,21]

HER2 mutations can be detected either by reverse-transcription polymerase chain reaction (RT-PCR) or by sequencing methods, such as next-generation sequencing. HER2 protein expression analysis could not be used as a surrogate marker for *HER2* mutations.[9,10]

HER2 *amplification and overexpression*

The identification and distinction between *HER2* amplification and overexpression remain debatable, probably due to the several available testing methods and the different definitions of HER2 positivity for each of them.[7,10]

Although not universally established, the most accepted definition for an *HER2* amplification is an average ratio of the *HER2* gene copy number to centromeres [*HER2*/chromosome enumeration probe 17 (CEP17)] that is ≥ 2 by fluorescent *in situ* hybridization (FISH).[22,23]

In the absence of a specific standard testing method for HER2 overexpression in NSCLC, the well-known immunohistochemistry (IHC) scoring system ranging between 0 and 3+ (with IHC 0-1+ defined as HER2 negative, IHC 2+ as weak to moderate and IHC 3+ as strong when staining in 10% of tumour cells), remains the most frequently used method to detect HER2 overexpression.[24,25]

Contrary to breast cancer where HER2 overexpression often occurs along with *HER2* amplification, this co-occurrence has not been confirmed in lung cancer.[7,9] The level of expression of HER2 in NSCLC cell lines is lower than in breast cancer, and the mechanism of overexpression is different. While breast cancer cell lines overexpress HER2 because of gene amplification in the majority of cases, this occurs infrequently in NSCLC and is more often attributable to polysomy (usually defined by an absolute *HER2* gene copy number higher than 5 or 6, but *HER2*/CEP17 <2).[24,25] Bunn *et al.* found a strong correlation between HER2 protein expression assessed by IHC and *HER2* gene copy number by FISH (32% of cell lines tested HER2 IHC positive: 26%, 2+; 5%, 3+); and polysomy determined by FISH was often found rather than true amplification.[26] Therefore, in case of HER2 2+ or 3+ expression, an additional FISH analysis needs to be carried out in order to discriminate between these possibilities.

Other parameters and different cut-offs have also been used to identify HER2 molecular alterations, hence the disparity between the different studies.[24,27,28] Table 1 summarizes the different testing methods for *HER2* mutations, amplification and overexpression.

TARGETING HER2 IN LUNG CANCER

Many prospective studies failed to identify an association between *HER2* amplification or overexpression and response to conventional chemotherapy.[29-31] Conversely, Wang *et al*. reported inferior outcomes in patients with *HER2*-mutant NSCLC who received pemetrexed-based chemotherapy compared with those with *ALK/ROS1* rearrangements, thus highlighting the need for effective HER2-targeting drugs in clinical practice.[32]

Table 1. Diagnostic methods of HER2 molecular alterations

	Main techniques	Alternative techniques
HER2 mutation	Sequencing techniques (NGS)	RT-PCR qPCR
HER2 amplification	FISH (*HER2*/CEP17>2)	NGS (copy number >6) ELISA (serum HER2 ECD >15 ng/ml) qRT-PCR (HER2 mRNA)
HER2 overexpression	IHC (2-3+)	qPCR and qRT-PCR (HER2 mRNA[a])

CEP17, chromosome enumeration probe 17; ECD, extracellular domain; ELISA, enzyme-linked immune assay; IHC, immunohistochemistry; mRNA, messenger RNA; NGS, next-generation sequencing; qPCR, quantitative polymerase chain reaction; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction; RT-PCR, reverse-transcriptase polymerase chain reaction.
[a] Brabender et al.[28] used a cut-off value of 1.8 to report that high HER2 mRNA levels were associated with an unfavourable prognosis in NSCLC with HER2 overexpression.

Many studies were conducted to evaluate the efficacy of anti-HER2 agents in patients with *HER2*-mutant or HER2-positive NSCLC (including *HER2* amplified or HER2 overexpressing). In contrast to breast and gastric cancers, these agents are still not considered a standard of care in lung cancer. Nevertheless, some recently published results are quite promising.

There are several methods for targeting HER2 molecular alterations, including small molecule TKIs, anti-HER2 antibodies as well as emerging ADCs. In the following section, we describe the available drugs targeting HER2 according to their mechanism of action and the corresponding HER2 molecular alteration.

Non-selective HER2 tyrosine kinase inhibitors

Table 2 summarizes the activity of HER2 TKIs in *HER2*-mutant NSCLC. Dual EGFR/HER2 TKIs such as afatinib and irreversible pan-HER TKIs like dacomitinib or neratinib have shown little activity against *HER2*-mutant refractory NSCLC, mainly in small phase II studies with ORRs ranging from 0% to 19%.[33-38]

Afatinib. Afatinib was first evaluated by De Grève *et al*. in a phase II study of patients with pre-treated NSCLC harbouring *EGFR* or *HER2* mutations. Upon progression, patients could continue afatinib 50 mg with the addition of paclitaxel (80 mg/m^2 weekly in 3/4-week cycles). Among seven patients with *HER2* mutations, one and five achieved unconfirmed partial response (PR) and disease control on afatinib monotherapy, respectively, while another patient had a confirmed PR of 41.9 weeks after receiving combination therapy. The most common afatinib-related adverse events (AEs) of any grade were diarrhoea (95%) and rash/acne (80%). Up to 20% of patients discontinued treatment and 44% required at least one dose adjustment due to AEs.[34]

In a compassionate use program, 28 heavily pre-treated patients with *HER2*-mutant NSCLC received afatinib 30-50 mg daily. Median time-to-treatment failure (TTF) was 2.9 months. Four out of 10 patients with the A775_G77GinsYVMA insertion remained on afatinib for >1

Table 2. HER2 tyrosine kinase inhibitors in NSCLC with *HER2* mutations

TKI	Study	Sample size (*n*)	Population	Main *HER2* mutations	Efficacy data				Safety and treatment modification			References
					ORR *n* (%)	DCR *n* (%)	Median PFS, months (95% CI)	Median OS, months (95% CI)	All grade AEs (%)	Grade 3-5 (%)	Dose reduction, discontinuation	
Afatinib	PhII Basket trial	7/41[a] (cohort 3)	29% ≥3 prior ChT lines	—	0/7 (0)	5/7 (71)	17 weeks (CI not reported)	—	Diarrhoea (95%), rash/acne (80%), stomatitis (46%)	—	20% discontinuation 44% dose reduction	De Grève *et al.*[34] *Lung Cancer*, 2015
	PhII Single arm	28	57% ≥3 prior ChT lines	A775_G776insYVMA (10) M774 (2)[b]	3/16 (19)	11/16 (69)	TTF 2.9 months	—	Diarrhoea (35.7%), skin disorders (28.6%), stomatitis (14.3%)	—	—	Peters *et al.*[36] *JTO*, 2018
	PhII Single arm NICHE trial	13	38% received afatinib as third line	A775_G776insYVMA (9)	1/13 (7.7)	7/13 (53.8)	15.9 weeks (6-35.4)	56 weeks (16.3-NR)	Rash, diarrhoea, vomiting	Dyspnoea (15.3%) One grade 5 acute renal injury	—	Dziadziuszko *et al.*[35] *JTO*, 2019
Dacomitinib	PhII Single arm	26/30[c]	83% ≥1 prior ChT lines	A775_G776insYVMA	3/26 (12)	—	3 (2-4)	9 (7-21)	Diarrhoea (90%),	Diarrhoea (23%)	13% discontinuation 17% dose reduction	

Continued

Table 2. Continued

TKI	Study	Sample size (*n*)	Population	Main *HER2* mutations	Efficacy data				Safety and treatment modification			References
					ORR *n* (%)	DCR *n* (%)	Median PFS, months (95% CI)	Median OS, months (95% CI)	All grade AEs (%)	Grade 3-5 (%)	Dose reduction, discontinuation	
				50% (13) G776delinsVC (2)					dermatitis (73%), fatigue (57%)			Kris *et al.*[33] *Ann Oncol*, 2015
Neratinib	PhII Basket trial SUMMIT study (NCT01953926)	26/141[d] (lung cohort)	46.4% ≥3 prior ChT lines	—	2/26 (3.8)	11/26 (42.3)	5.5 (CI not reported)	—	Diarrhoea (73.8%), nausea (43.3%), vomiting (41.1%)	Diarrhoea 22%	2.8% discontinuation	Hyman *et al.*[37] *Nature*, 2018
	PhII PUMA-NER-420 study Neratinib (N) ± temsirolimus (T) (NCT01827267)	60[e]	—	Exon 20 insertion 93.5%	0/17 (0) (N) *versus* 8/43 (19) (NT)	6/17 (35) *versus* 22/43 (51)	3 (1.4-6.9) *versus* 4.1 (2.9-5.6)	10.0 (4.9-19) *versus* 15.8 (10.8-19.5)	Diarrhoea (82% *versus* 86%) Stomatitis (6% *versus* 49%)	Diarrhoea (12% *versus* 14%), stomatitis (0% *versus* 7%)	—	Gandhi *et al.*[38] *JCO*, 2017
Poziotinib	Ph II (NCT03066206)	12	—	Y772dupYVMA (9) or G778dupGSP (3)	5/12 (42)	10/12 (83)	5.6 (CI not reported)	—	Dry skin (77%), paronychia (77%), mucositis (77%), diarrhoea (69%)	Diarrhoea (17%) and rash (58%)	67% dose reduction No discontinuation	Robichaux *et al.*[48] *Cancer Cell*, 2019
	PhII Basket trial ZENITH20 study (NCT03318939)	90 (cohort 2)	67% ≥2 prior lines	—	25/90 (27.8)	63/90 (70)	5.5 (3.9-5.8)	—	—	Rash (29%) Diarrhoea (26%) Stomatitis (10%)	87% dose reductions 14% discontinuation	Cornelissen *et al.*[51] *WCLC* 2020
Pyrotinib	Ph I-II (NCT02535507)	15	Median number of prior lines 2 (1-5)	A775_G776insYVMA (67%)	8/15 (53.3)	11/15 (73.3)	6.4 (1.6-11.2)	12.9 (2.1-23.8)	Diarrhoea (27%) Anaemia (27%) Hypocalcaemia (27%)	None	None	Wang *et al.*[52] *Ann Oncol*, 2019.
	PhII Single arm (NCT02834936)	60	41.7% ≥2 prior ChT lines 25% prior targeted therapy	12-bp exon 20 ins (73.3%) G776 (10%) 9-bp exon 20 ins (8.3%)	18/60 (30)	51/60 (85)	6.9 (5.5-8.2)	14.4 (12.3-21.3)	Diarrhoea (91.7%) Elevated blood creatinine (28.3%) Vomiting (28.3%) Elevated liver enzymes (30%)	Diarrhoea (20%)	5% dose reduction 1.7% discontinuation	Zhou *et al.*[54] *JCO*, 2020
Tarloxotinib	PhII Basket trial RAIN-701 study (NCT03805841)	11/23[f] (cohort B)	Progressive disease after platinum-based ChT	—	2/9 (22)	6/9 (67)	—	—	QT prolongation (60.9%) Rash (43.5%) Diarrhoea (21.7%) Nausea (21.7%)	QT prolongation (34.8%) Rash (4.3%) Diarrhoea (4.3%)	21.7% dose reduction 4.3% discontinuation	Liu *et al.*[57] ESMO 2020

AEs, adverse events; ChT, chemotherapy; CI, confidence interval; DCR, disease control rate; ORR, objective response rate; OS, overall survival; Ph, phase; PFS, progression-free survival; TKI, tyrosine kinase inhibitor; TTF, time-to-treatment failure.

[a] Three cohorts (1 and 2: *EGFR* mutation and increased copy number of *EGFR* by FISH; 3: *HER2* mutations); 33 patients received afatinib and 8 subsequently received afatinib plus paclitaxel (3 of them in the cohort 3).

[b] Data only available in 12 out of 28 patients.

[c] Twenty-six cases with *HER2* mutation and 4 with *HER2* amplification; in the latter, ORR was 0%.

[d] Sixteen harbour *HER3* and 125 *HER2* mutations, including different solid tumours; the most common being breast, lung, bladder and colorectal cancer.

[e] Seventeen patients received neratinib, and 43 patients the combination of neratinib with temsirolimus.

[f] Three cohorts (A: *EGFR* exon 20 insertion, B: *HER2*-activating mutation and C: solid tumours harbouring *NRG1, EGFR, HER2* or *HER4* fusions). In cohort B, only 9 of 11 patients were evaluable for response.

year, with a TTF of 9.6 months, an ORR of 33% and a disease control rate (DCR) of 100%.[36] These results suggest that afatinib may be effective in patients with this particular mutation subtype. Similar findings were reported in a retrospective study where two of three PRs were observed in patients carrying the A775_G776insYVMA insertion.[39] Conversely, in another observational study, patients with G778_P780dup and G776delinsVC mutations derived the greatest benefit from afatinib [ORR 40% and median progression-free survival (PFS) of 7.6 months], whereas those with the A775_G776insYVMA insertion ($n = 14$) did not respond.[40]

In the EUHER2 retrospective study, 65 out of 101 patients with *HER2*-mutant NSCLC were treated with anti-HER2 drugs. Afatinib had modest activity in 11 patients, with an ORR and a median PFS of 18.2% and 3.9 months, respectively.[39,41]

Lately, the NICHE phase II trial included 13 patients with *HER2*-mutant refractory NSCLC treated with afatinib 40 mg daily. ORR and DCR were 7.7% and 53.8%, respectively. Median PFS and median overall survival (OS) times reached 15.9 weeks [95% confidence interval (CI), 6-35.4 weeks] and 56.0 weeks [95% CI, 16.3 weeks-not reached (NR)], respectively. The toxicity profile was generally consistent with that of previous studies. Grade 3-4 AEs were uncommon (<10% of patients).[35]

Dacomitinib. Dacomitinib is an irreversible pan-HER2 TKI that binds to EGFR, HER2 and HER4 tyrosine kinases. In a prespecified cohort from a phase II trial that included pre-treated *HER2*-mutant patients receiving dacomitinib 30-45 mg daily, 3 of 26 patients achieved a PR (ORR 12%). Median PFS and OS were 3 (95% CI, 2-4) months and 9 (95% CI, 7-21) months, respectively. All responders had either a P780_Y781insGSP or an M774delinsWLV mutation, whereas no responses were observed in patients with the A775_G776insYVMA insertion.[33] The most common treatment-related AEs of any grade were diarrhoea (90%, one patient developed grade 4 diarrhoea) and skin rash (73%). Seventeen percent of patients required dose reduction, and 13% stopped dacomitinib due to toxicity.[33]

Neratinib. Like dacomitinib, neratinib irreversibly binds to EGFR, HER2 and HER4. Nagano *et al*. reported *in vitro* activity of both afatinib and neratinib against the A775_G776insYVMA insertion in patient-derived tumour organoids with higher levels of cell death. In contrast, L775P and L775S mutations were associated with resistance to these agents.[42]

The SUMMIT phase II basket trial included 26 refractory NSCLC cases harbouring *HER2* mutations treated with neratinib 240 mg daily. A PR was observed in one patient only (ORR 3.8%), who turned out to have a kinase domain missense mutation (L755S). Despite the low response rate, median PFS was 5.5 months, with six patients remaining on therapy for >1 year.[37] Diarrhoea (73.8%), nausea (43.3%) and vomiting (41.1%) were the most common any grade AEs. Grade 3 or 4 events were mainly represented by diarrhoea (22% of cases).[37]

Based on promising preclinical data on the combination of neratinib with a mechanistic target of rapamycin (mTOR) inhibitor, a phase II study randomized patients to receive neratinib 240 mg daily with or without weekly intravenous temsirolimus (8 mg) (the dose was escalated to 15 mg weekly following a 3-week cycle if well tolerated).[38,43,44] In the expansion cohort of 62 patients, objective responses were reported in 8 of 43 patients in the combination arm (ORR 19% *versus* 0% in the monotherapy arm). Patients treated with neratinib and temsirolimus had an increased incidence of stomatitis (49% *versus* 6%) and comparable rates of diarrhoea (86% *versus* 82% any grade, 14% *versus* 12% grade 3).[38]

Selective HER2 tyrosine kinase inhibitors

Recently, novel, more selective and structurally advantageous pan-HER2 TKIs have been developed with the objective of improving outcomes in NSCLC with *HER2* mutations (Table 2).

Poziotinib. Poziotinib is a covalent, irreversible and potent EGFR/HER2 inhibitor. Like afatinib, it is a quinazoline derivative, with a smaller size and more flexible structure in order to circumvent the hindered binding pocket of exon 20 insertions. *In vitro* and in patient-derived xenograft (PDX) models with *HER2* exon 20 mutant NSCLC, poziotinib appeared to be more effective than other pan-HER2 TKIs,[45,46] showing an average half-maximal inhibitory concentration (IC_{50}) value of 1.9 nM in Ba/F3 cell lines, thus becoming 200 times and 6 times more potent than osimertinib and afatinib, respectively.

A first-in-human phase I trial examined the safety and maximum tolerated dose (MTD) of continuous and intermittent poziotinib in 75 patients with advanced, genomically unselected, solid tumours including NSCLC.[47] Treatment was well tolerated, with a recommended phase II dose of 16 mg daily. Eight out of 51 patients in the continuous dosing cohort and 4 out of 20 patients in the intermittent dosing one achieved PRs, hence supporting further clinical development of poziotinib.[47]

Early results from a single-centre phase II trial of poziotinib in 12 heavily pre-treated NSCLC patients with *EGFR* or *HER2* exon 20 mutations demonstrated an ORR of 42% (with the 16 mg daily dose), with durations of response exceeding 1 year, and a median PFS time reaching 5.6 months in the *HER2*-mutant group.[48,49] All the included patients had either an Y772dupYVMA or a G778dupGSP insertion. Overall, the safety profile of poziotinib was similar to that of other *EGFR* TKIs with eight patients experiencing grade 3-4 AEs, the majority of which were diarrhoea (17%) and rash (58%). Sixty-seven percent of patients required at least one dose reduction, but none of them discontinued the treatment due to toxicity.[48]

ZENITH20 is a currently ongoing confirmatory multicentre and multicohort phase II trial of poziotinib that includes treatment-naïve patients.[50,51] Results from the cohort including 90 *HER2*-mutant pre-treated NSCLC patients were recently presented at the World Conference on Lung Cancer

(WCLC) 2020, showing an ORR of 27.8% (95% CI, 18.9%-38.2%), a median duration of response (DoR) of 5.1 months (95% CI, 4.2-5.5 months) and a median PFS of 5.5 months (95% CI, 3.9-5.8 months).[51] Greater responses (38.7%) were observed in patients who were heavily pre-treated (≥3 prior treatment lines). Four out of 14 patients with CNS involvement at baseline achieved objective responses (28.6%) while the rest of them remained stable, hence resulting in a CNS-specific DCR of 100%.[48,50,51]

The most common AEs of any grade as well as grade ≥3 events were rash (29%), diarrhoea (26%) and stomatitis (10%). They led to dose reductions and permanent treatment discontinuation in 87% and 14% of patients, respectively.[50,51]

Pyrotinib. Pyrotinib, a 3-cyanoquinoline derivative, is a small-sized covalent pan-HER inhibitor of EGFR, HER2 and HER4. It was found to be superior to afatinib and trastuzumab—emtansine (T-DM1) both in *in vitro* and *in vivo* studies on NSCLC patient-derived organoids and PDX murine models harbouring *HER2* mutations.[52] Drug response curves of the organoids treated with afatinib and pyrotinib had comparable IC_{50} levels (112.5 and 89.1 nM, respectively); however, according to plasma concentrations from previous phase I trials, pyrotinib achieved a significantly higher inhibition of cell growth.[53] Of note, pyrotinib-treated mice displayed significant reduction of tumour burden (−52.2%) when compared to afatinib or T-DM1-treated mice (−25.4% and −10.9%, respectively).[52]

Results from a single-centre phase II study of 15 pre-treated *HER2*-mutant NSCLC patients receiving pyrotinib 400 mg daily were favourable, with 8 out of 15 patients achieving a PR (ORR 53.3%) and with a median PFS of 6.4 months (95% CI, 1.6-11.2 months).[52] Updated data from the subsequent multicentre phase II trial including 60 patients with *HER2*-mutant refractory NSCLC revealed an ORR of 30%, with a median DoR of 6.9 months (95% CI, 4.9-11.1 months) and median PFS and OS of 6.9 months (95% CI, 5.5-8.3 months) and 14.4 months (95% CI, 12.3-21.3 months), respectively.[54,55] In a prespecified subgroup analysis, higher ORRs were obtained in 44 patients harbouring a 12-bp exon 20 insertion (27.3%) and in 5 patients with a 9-bp exon 20 insertion (60%), whereas in patients with G776 and L755P mutations ORRs were 16.7% and 25%, respectively. Response rates were also higher in patients pre-treated with at least two lines of chemotherapy (ORR 44% versus 20%); yet no differences were noted when it came to CNS involvement (25% versus 31.3%).[54]

With pyrotinib, the most frequent AEs of any grade were diarrhoea (91.7%), elevated blood creatinine (30%) and vomiting (28.3%). Grade ≥3 AEs occurred in 28.3% of patients (diarrhoea, mainly) and led to dose adjustment and early treatment discontinuation in 5% and 1.7% of the patients, respectively.[54]

Tarloxotinib. Tarloxotinib is a hypoxia-activated prodrug of a pan-HER kinase inhibitor that releases a potent irreversible active metabolite (tarloxotinib-E) under hypoxic conditions. It is also an *NRG1* fusion inhibitor that in turn activates HER2 and HER3. *In vivo* assays have shown tarloxotinib-induced tumour regression in murine xenograft models with NSCLC harbouring *EGFR* exon 20 insertions and HER2 alterations. Pharmacokinetic analysis confirmed markedly higher levels of tarloxotinib-E in tumour tissue compared to plasma or skin; and eventually, one patient with lung adenocarcinoma carrying the *EGFR* exon 20 A775_G776insYVMA insertion had a dramatic clinical response to tarloxotinib.[56]

A phase II trial is currently recruiting chemotherapy pre-treated NSCLC patients harbouring *EGFR* exon 20 insertions or *HER2* mutations, as well as patients with any solid tumour and *NRG1*, *EGFR*, *HER2* or *HER4* fusions. Tarloxotinib is being administered intravenously at a dose of 150 mg/m^2 weekly. Preliminary data from the *HER2*-mutant cohort (n = 11) revealed two PRs (ORR 22%) and four stable diseases (DCR 67%) out of nine assessable patients. Most AEs were of grades 1 or 2, the most reported ones being prolonged QTc (60.9%; 34.8% grade 3-4), rash (43.5%), nausea (21.7%) and diarrhoea (21.7%). Twenty-two percent and 4.3% of patients required dose reductions and discontinued tarloxotinib, respectively.[57]

Mobocertinib. Mobocertinib (TAK-788/AP3278) is a next-generation TKI that irreversibly binds to EGFR via a covalent modification of Cys797 residue in the EGFR active site. Activity of AP32788 was assessed in Ba/F3 cell lines that were engineered to express mutant variants of *EGFR* and *HER2*. In contrast to erlotinib, gefitinib and afatinib, AP32788 inhibited all mutant variants of both *EGFR* (IC_{50} 2.4-22 nM) and *HER2* (IC_{50} 2.4-26 nM) more potently than wild-type *EGFR* (IC_{50} 35 nM).

Early results of a phase I/II first-in-human multicentre study of 34 refractory NSCLC patients with *EGFR/HER2* exon 20 insertions reported objective responses in 3 out of 14 assessable patients, all of them harbouring an *EGFR* exon 20 insertion.[58] The recommended dose was 160 mg daily and toxicity was consistent with other TKIs. The phase II trial is still recruiting patients to evaluate the efficacy of TAK-788 in patients with NSCLC harbouring *EGFR* or *HER2* exon 20 mutations (NCT02716116). While efficacy data in the *HER2*-mutant subtype are still awaited, updated results from the *EGFR*-mutant expansion cohort have recently been published, with confirmed PRs seen in 12 of 28 patients (ORR 43%), DCR of 86% and median PFS of 7.3 months (95% CI, 4.4-15.6 months).[59] The most common AEs of any grade were diarrhoea (82%), rash (46%), nausea (39%), anorexia (39%) and vomiting (36%). Grade 3-4 AEs were reported in up to 5% of patients, with diarrhoea being the most commonly reported grade 3 AE.[59]

Monoclonal antibodies against HER2

Trastuzumab is a monoclonal immunoglobulin G1 humanized murine antibody that binds to the extracellular IV domain of the HER2 receptor and therefore blocks its dimerization. It promotes receptor internalization and/or degradation and eventually inhibits the PI3K/AKT signalling pathway. Moreover, *in vitro* assays have shown that trastuzumab can also trigger cell-mediated cytotoxicity.[60]

Trastuzumab-containing chemotherapy regimens are a gold standard in the management of advanced breast and gastric cancers with *HER2* amplification or overexpression.[61-64] Ever since the first published paper by Cappuzzo and colleagues demonstrating a sustained response to trastuzumab-based chemotherapy in a heavily pre-treated patient with advanced *HER2*-mutant NSCLC, several HER2 targeting strategies have been tested in HER2-altered NSCLC patients with variable outcomes.[65]

In relapsed NSCLC with *HER2* mutations, a retrospective study of 57 patients reported an ORR of 50% and a median PFS of 4.8 months (95% CI, 3.4-6.5 months) in the chemotherapy and trastuzumab combination group.[41] In contrast, the only available phase II trial of trastuzumab monotherapy in *HER2*-mutant refractory NSCLC included seven patients and failed to achieve objective responses although DCR and median PFS time were 70% and 5.2 months (95% CI, 1.4-6.3 months), respectively.[66] The previous results remain questionable in the absence of larger prospective randomized studies.

In HER2-positive refractory NSCLC, a lack of response and/or benefit from trastuzumab ±chemotherapy compared to chemotherapy alone has been consistently observed regardless of the HER2 'level' of positivity as determined by IHC or FISH.[67-69] Conversely, in another phase II trial of 101 patients with *HER2*-amplified or HER2-overexpressing untreated NSCLC, the addition of trastuzumab to cisplatin and gemcitabine seemed beneficial in IHC3- or FISH-positive cases ($n = 12$; with an ORR of 83% and a median PFS of 8.5 months). However, the sample size was too small to draw further conclusions and no differences between the two treatment arms were found in the *HER2*-amplified/overexpressing overall population [ORR of 41% *versus* 36% and median PFS of 7 (95% CI, 6-7.7 months) *versus* 6.1 months (95% CI, 0.1-19.6 months)].[69] The toxicity profile was comparable between treatment arms (mainly gastrointestinal and haematologic AEs), with the exception of a >15% decrease in the left ventricular ejection fraction in 3 out of 50 patients who received trastuzumab.[69]

The combination of trastuzumab with pertuzumab, a recombinant humanized monoclonal antibody that specifically targets the HER2 dimerization domain thus blocking ligand-dependent heterodimerization of HER2 with other HER family members, has shown limited activity in the phase IIa MyPathway basket trial, which included 30 patients with *HER2*-mutant or HER2-positive refractory NSCLC (ORR of 21% and 13%, respectively).[70]

Table 3 summarizes the latest findings about monoclonal antibodies in HER2-altered NSCLC.

Antibody–drug conjugates against HER2

ADCs are emerging antitumour agents that combine the unique binding capacities of monoclonal antibodies with the cytotoxic activity of chemotherapy to specifically target and harm tumour cells. In addition, they can also stimulate the immune cell effector function and disrupt receptor dimerization. Following their success mainly in breast and gastric cancers, these agents are currently being evaluated in HER2-altered NSCLC.[71-76]

Trastuzumab–emtansine. T-DM1 is an anti-HER2 ADC composed of trastuzumab and the cytotoxic microtubule agent emtansine (DM1), a maytansine derivative. T-DM1 penetrates into HER2-positive cells through receptor-mediated endocytosis and DM1 is released after proteolytic degradation of the antibody moiety in the lysosomes.[77]

A small phase II trial reported limited efficacy of T-DM1 monotherapy in 15 relapsed HER2-altered NSCLC patients with a global ORR of 6.7% and median PFS and OS times of 2 (95% CI, 1.4-4) months and 10.9 (95% CI, 4.4-12) months, respectively. No responses were obtained in the *HER2*-amplified/overexpressing subgroup, and only one of seven patients in the *HER2*-mutant cohort had a PR (ORR 14.3%). This study was terminated early because of the limited efficacy.[78]

Later on, analyses from a phase II basket trial by Li and colleagues highlighted the potential role of T-DM1 in *HER2*-mutant NSCLC patients, administered at the dose of 3.6 mg/kg intravenously. Eight out of 18 patients experienced PRs, with a median DoR of 4 months (range, 2-9 months) and a median PFS of 5 months (95% CI, 3-9 months).[79] Updated data that included 28 patients with *HER2*-mutant pretreated NSCLC showed an ORR of 50% (95% CI, 31% to 69%).[79-81]

T-DM1 was also administered to 11 patients with *HER2*-amplified NSCLC included in the latter basket trial and achieved an ORR of 55% (95% CI, 23%-83%).[79-81] Moreover, Peters *et al*. studied the efficacy of T-DM1 in HER2-overexpressing NSCLC, with no responses in the IHC2+ subgroup and an ORR of 20% (95% CI, 5.7%-43.7%) in the IHC3+ subgroup despite comparable median PFS (2.6 versus 2.7 months) and OS (12.2 versus 5.3 months) times. However, when HER2 overexpression patterns were further analysed, three of four responders had *HER2* amplification and two patients had a concomitant *HER2* mutation. In conclusion, this study showed that the HER2 positivity status determined solely by IHC cannot be a predictive biomarker for T-DM1 activity.[82]

T-DM1-related AEs were mainly of grades 1 or 2, including increased liver transaminases (63%), thrombocytopenia (31%) and nausea (29%). No dose reductions or treatment discontinuation due to toxicity were needed in the *HER2*-mutant cohort.[79]

Trastuzumab–deruxtecan. Trastuzumab–deruxtecan (T-Dxd or DS-8201a) is a novel HER2-targeting ADC composed of trastuzumab, an enzymatically cleavable peptide linker and a novel topoisomerase I inhibitor called MAAA-1181. Its mechanism of action differs from other ADCs: it binds to topoisomerase I–DNA complexes and stabilizes them which in turn induces DNA double-strand breaks and apoptosis.[83] T-Dxd's stable and homogeneous design, despite its higher drug-to-antibody ratio compared to other available ADCs (8 *versus* 2-4), allows for a steady delivery of the topoisomerase I inhibitor in HER2-low expressing conditions. In addition, its high membrane permeability facilitates its diffusion even across HER2-negative cells.[84,85]

Table 3. Monoclonal antibodies in NSCLC with HER2 molecular alterations

Agent	Study	Sample size (*n*)	Population	HER2 alteration type	Efficacy data				Safety and treatment modification			References
					ORR *n* (%)	DCR *n* (%)	Median PFS, months (95% CI)	Median OS, months (95% CI)	All grade AEs (%)	Grade 3-5 (%)	Dose reduction, discontinuation	
Trastuzumab ± ChT	PhII CDDP (C) - gemcitabine (G) ± trastuzumab (T)	101 (51 CG and 50 CGT)	Treatment naïve	*HER2* amplification/ overexpression (IHC2-3+, *HER2*/ CEP17≥2 by FISH, >15 ng/ml HER2 ECD by ELISA)	21/51 (41) *versus* 18/ 50 (36)	48/51 (94) *versus* 40/ 50 (80)	7 (6-7.7) *versus* 6.1 (0.1-19.6)	NR (1-NR) *versus* 12.2 (0.1-19.6)	Nausea 52% *versus* 74% Vomiting 38% *versus* 46% Fatigue 40% *versus* 42% Anaemia 40% *versus* 36% Decreased LVEF 0% *versus* 6%	Neutropenia 58% *versus* 57% Thrombocytopenia 34% *versus*35%	One patient in the CGT arm discontinued treatment due to decreased LVEF	Gatzemeier *et al.*[69] *Ann Oncol*, 2004
	PhII Single arm ECOG 2598 study CBDCA-paclitaxel + trastuzumab	53	Treatment naïve	HER2 overexpression (IHC1-3+)	13/53 (25)	—	3.3 (CI not reported)	10.1 (6.7-14.6)	Anaemia 91% Fatigue 64% Sensory neuropathy 58% Nausea 55% Decreased LVEC 7%	Neutropenia 34% Thrombocytopenia 16% Sensory neuropathy 7%	—	Langer *et al.*[68] *JCO*, 2004
	Retrospective study EURHER2 cohort	57/101[a]	Median number of prior lines 3 (1-11)	*HER2* mutation (exon 20 insertions; subtypes not specified)	29/57 (51)	43/57 (76)	4.8 (3.4-6.5)	13.3 (8.1-15)	—	—	—	Mazieres *et al.*[41] *Ann Oncol*, 2016
	Ph II Single arm HOT1303-B trial	10	Median number of prior lines 3 (2-6)	*HER2* mutation ($n = 7$) (4 A755_ G776insYVMA, 1 G776>VC, 2 S310F) *HER2* amplification/ overexpression ($n = 3$) (IHC 3+ or IHC2+/ DISH+)	0/15 (0)	11/15 (70)	5.2 (1.4-6.3)	—	—	—	—	Kinoshita *et al.*[66] ESMO 2018
Trastuzumab—pertuzumab	PhII Basket trial MyPathway (NCT02091141)	14/30	Median number of prior lines 2.5 (0-9)	*HER2* mutation (exon 20 insertions, deletions in amino acids 755-759, and nonsynonymous amino acid substitutions)	3/14 (21)	6/13 (43)	—	—	—	—	—	Hainsworth *et al.*[70] *JCO*, 2018
		16/30		*HER2* amplification/ overexpression (IHC3+, *HER2*/ CEP17 >2 by FISH, copy number >6/increased by NGS)	2/16 (13)	4/16 (25)	—	—				

AEs, adverse events; CBDCA, carboplatin; CDDP, cisplatin; CEP17, centromeric probe for chromosome 17; ChT, chemotherapy; CI, confidence interval; DISH, dual colour *in situ* hybridization; DCR, disease control rate; ECD, extracellular domain; FISH, fluorescence *in situ* hybridization; IHC, immunochemistry; LVEF, left ventricular ejection fraction; NR, not reached; ORR, objective response rate; OS, overall survival; Ph, phase; PFS, progression-free survival; TTF, time-to-treatment failure. .

[a] Fifty-five of 101 patients were treated with trastuzumab in combination with chemotherapy (including vinorelbine, docetaxel, paclitaxel and cisplatin) and 2 patients received trastuzumab alone.

In vitro and *in vivo* assays of T-Dxd showed excellent antitumour activity against T-DM1-resistant and HER2-low expression models, along with a favourable pharmacokinetic and safety profile, thus suggesting its potential to address unmet medical needs in patients with HER2-altered tumours.[84]

The expansion cohort of the first-in-human phase I trial evaluating T-Dxd in non-breast and non-gastric/gastro-oesophageal tumours enrolled patients with relapsed NSCLC harbouring HER2 alterations. T-Dxd demonstrated promising antitumour activity with an acceptable safety profile; the MTD was 6.4 mg/kg intravenously every 3 weeks.[86] Updated data including 11 patients with *HER2*-mutant pre-treated NSCLC showed an ORR of 72.7%, a median DoR of 9.9 months (95% CI, 6.9-11.5 months) and a median PFS of 11.3 months (95% CI, 8.1-14.3 months).[87]

Recently, results from the multicentre phase II trial, DESTINY-Lung01, evaluating the efficacy of T-Dxd in refractory NSCLC harbouring HER2 molecular alterations, have been presented at the American Society of Clinical Oncology 2020 and WCLC 2020 meetings. The *HER2*-mutant cohort (cohort 2) included 42 patients, yielded unprecedented efficacy data with an ORR of 61.9% (95% CI, 45.6%-76.4%), a median DoR that was NR (95% CI, 5.3 months-NR) and a median PFS of 14 months (95% CI, 6.4-14 months). OS data were still immature (95% CI, 11.8 months-NR).[88,89]

The DESTINY-Lung01 trial also included 49 patients with HER2-overexpressing NSCLC (cohort 1). Results were promising albeit less spectacular in comparison with cohort 2, with an ORR of 24.5% (95% CI, 13.3%-38.9%), a median DoR of 6 months (95% CI, 3.2 months-NR) and a median PFS of 5.4 months (95% CI, 2.8-7 months). Responses did not differ according to HER2 IHC expression levels (ORR 20.0% *versus* 25.6% in IHC3+ and IHC2+ patients, respectively).[89]

Regarding T-Dxd toxicity, gastrointestinal and haematological events were the most common AEs of any grade, with neutropenia being the most common grade $\geq$3 AE. In the phase II study, up to 52%-55% of AEs were of grade 3 or higher and 22%-24% led to treatment discontinuation.[88,89] Importantly, five cases of T-Dxd-related interstitial lung disease (ILD) were reported in the phase I basket trial. Three of these patients required hospitalization and one died of respiratory failure; of note, the latter patient had a history of ongoing dyspnoea and had initially undergone pneumonectomy.[87] In the phase II DESTINY-Lung01 trial, five patients in the *HER2*-mutant cohort presented with grade 2 drug-related ILD. In the HER2-overexpressing cohort, drug-related ILD occurred in 16.3% of patients (three patients with grade 5, three with grade 2 and two with grade 1).[88] All patients with grade 5 ILD had received prior immune-checkpoint inhibitors (ICIs). In summary, ILD remains a serious identified risk for patients treated with T-Dxd and requires careful monitoring and multidisciplinary management. Further investigations will be needed when more follow-up data become available.

Table 4 summarizes data about ADCs in HER2-altered NSCLC.

IMMUNE-CHECKPOINT INHIBITORS AND HER2 ALTERATIONS

Evidence on the use of ICIs in NSCLC patients with HER2 alterations remains scarce and is entirely based on retrospective studies listed in Table 5.[90-93]

The first results came from a cohort of 122 patients with *HER2*-mutant NSCLC, 26 of whom were treated with ICIs at the Memorial Sloan Kettering Cancer Centre. The ORR was 12%, with a median DoR of 3.4 months (range, 1.4-21.2 months). None of the responders carried the A775_G776insYVMA insertion. PFS and OS times were 1.9 (95% CI, 1.5-4 months) and 10.4 months (95% CI, 5.9 months-NR), respectively.[90]

Next, the IMMUNOTARGET registry retrospectively evaluated the role of ICIs in 551 patients carrying different molecular alterations. Twenty-nine patients had *HER2*-mutant NSCLC and achieved an ORR of 7.4% with a median PFS of 2.5 months (95% CI, 1.8-3.5 months). In certain driver subgroups, such as *HER2* mutations, PFS was positively correlated with the smoking status (3.4 months in smokers *versus* 2 months in non-smokers; $P = 0.04$). Of note, the median percentage of programmed death-ligand 1 (PD-L1) expression was 0% in the *HER2*-mutant cohort.[91]

More recently, the French Lung Cancer Group (GFPC) reported objective responses to ICIs in 6 out of 23 patients with *HER2*-mutant relapsed NSCLC (ORR 27.3%), with a median DoR of 15.2 months (95% CI, 7 months-NR). Survival data were in line with previous reports, with a median PFS of 2.2 months (95% CI, 1.7-15.2 months) and a median OS of 20.4 months (95% CI, 9.3 months-NR).[93]

These retrospective studies do not encourage the use of ICIs as a therapeutic strategy in patients with *HER2*-mutant NSCLC, much like those with *EGFR* and *ALK* alterations. Large prospective studies are needed to assess the true activity of ICIs in these subsets of patients.

CURRENT OBSTACLES AND FUTURE PERSPECTIVES

The considerable biological and clinical heterogeneity of NSCLC with HER2 molecular alterations may explain the current limited and heterogeneous activity of HER2-targeted therapies in NSCLC. Most of the studies evaluating anti-HER2 drugs in patients with *HER2*-mutant and HER2-positive NSCLC are small and do not distinguish between the three types of HER2 alterations, which impairs the interpretation of their predictive value in NSCLC. Standardization of HER2 detecting methods is mandatory and larger randomized studies are needed.

Also, although some preliminary data concerning CNS activity with novel TKIs have already been published and since brain metastases are common at least in *HER2*-mutant NSCLC, CNS activity should be further evaluated in future trials.[51,54]

In the near future, a paradigm shift from monotherapies towards combinations of agents with distinct/synergistic mechanisms of action, such as the combination of ADCs with irreversible TKIs or with ICIs, will likely change the therapeutic landscape of HER2-driven NSCLC.

Table 4. Antibody-drug conjugates in NSCLC with HER2 molecular alterations

Agent	Study	Sample size (*n*)	Population	HER2 alteration type	Efficacy data				Safety and treatment modification			References
					ORR *n* (%)	DCR *n* (%)	Median PFS, months (95% CI)	Median OS, months (95% CI)	All grade AEs (%)	Grade 3-5 (%)	Dose reduction, discontinuation	
Trastuzumab—emtansine	PhII Single arm	7/15	Median number of prior lines 4 (1-7)	*HER2* mutation (5 A775_G776insYVMA)	1/7 (4.3)	5/7 (71.4)	2.0 (1.2-4)	10.9 (4.4-12)	Interstitial pneumonia (7%)	Thrombocytopenia (40%), hepatotoxicity (20%), acute renal failure (7%)	—	Hotta *et al.*[78] *JTO*, 2018
		8/15		*HER2* amplification/ overexpression (IHC3+ or IHC2+ confirmed by FISH)	0/8 (0)	3/8 (37.5)						
	PhII Single arm	49	98% ≥1 prior ChT line	HER2 overexpression (IHC2-3+)	IHC2+: 0/29 (0) IHC3+: 4/20 (20)	IHC2+: 8/29 (28) IHC3+: 8/20 (40)	IHC2+: 2.6 (1.4-2.8) IHC3+: 2.7 (1.4-8.3)	IHC2+: 12.2 (3.8-23.3) IHC3+: 15.3 (4.1-NR)	Infusion reaction (14%), peripheral neuropathy (14%), haemorrhage (14%)	Infusion reaction (2%), thrombocytopenia (2%)	4% discontinuation	Peters *et al.*[82] *CCR*, 2019
	PhII Basket trial[a] (NCT02675829)	28/49	Median line of therapy for T-DM1 2 (1-7)	*HER2* mutation (subtypes not specified)	14/28 (50)	—	5 (3.5-5.9)	—	Hepatotoxicity (63%), thrombocytopenia (31), nausea (29%), fatigue (16%)	Thrombocytopenia (2%), anaemia (2%)	None	Li *et al.*[80,81] *JTO,* 2018 *Cancer Discov,* 2020
		11/49		*HER2* amplification (NGS, FISH)	6/11 (55)	—						
Trastuzumab—deruxtecan	Ph I (NCT02564900)	11/60[b]	Median number of prior lines 4 (1-10)	*HER2* mutation (44.4% exon 20 insertions)	8/11 (72.7)	10/11 (90.9)	11.3 (8.1-14.3)	17.3 (17.3-NR)	Nausea (74.6%), vomiting (52.6%), anaemia (39%), thrombocytopenia (37.4%)	Anaemia (25.4%), Neutropenia (20.3%), thrombocytopenia (15.3%), pneumonitis (1.7%)	23.7% dose reduction 8.5% discontinuation	Tsurutani *et al.*[87] *Cancer Discov,* 2020
	PhII Two-cohort and two-arm[c] DESTINYLung01 (NCT03505710)	42	Median number of prior lines 2 (1-6)	*HER2* mutation (38 mutations in the kinase; subtype not specified)	26/42 (61.9)	38/42 (90.5)	14 (6.4-14)	NR (11.8-NR)	Pneumonitis (11.9%)	Neutropenia (26.2%), anaemia (16.7%)	38.1% dose reduction 23.8% discontinuation	Smit *et al.*[88] ASCO 2020 Nakagawa *et al.*[89] WCLC 2020
		49	Median number of prior lines 3 (1-8)	HER2 overexpression (IHC2-3+)	12/49 (24.5)	34/49 (69.4)	5.4 (2.8-7)	11.3 (7.8-NR)	Pneumonitis (10.2%)	Neutropenia (20.4%) Pneumonitis grade 5 (2%)	32.7% dose reduction 12.2% discontinuation	

AEs, adverse events; ChT, chemotherapy; CI, confidence interval; DCR, disease control rate; FISH, fluorescence *in situ* hybridization; IHC, immunochemistry; NR, not reached; ORR, objective response rate; OS, overall survival; Ph, phase; PFS, progression-free survival; TTF, time-to-treatment failure.

[a] Ten patients presented concurrent *HER2* mutation and amplification, showing an ORR of 50%.

[b] Total of 60 patients including solid non-breast and non-gastric cancers, mainly colorectal (33.3%).

[c] Evaluating, in both cohorts, trastuzumab—deruxtecan at 5.4 mg/kg versus 6.4 mg/kg.

Table 5. Retrospective studies evaluating the efficacy of immune checkpoint inhibitors in NSCLC with *Her2* mutations

	Sample size, *n*	Type of ICIs and treatment line	PD-L1 expression ≥1%	ORR *n* (%)	DCR *n* (%)	Median PFS, months (95% CI)	Median OS, months (95% CI)	Reference
MSKCC	26	Not specified	23%	3/26 (12)		1.9 (1.5-4)	10.4 (5.9-NR)	Lai *et al.*[90] ASCO 2018
IMMUNOTARGET registry[a]	29	Nivolumab 89.6% ≥2 lines 94.5%	53.3%	2/29 (7.4)	9/29 (31)	2.5 (1.8-3.5)	20.3 (7.8-NR)	Mazieres *et al.*[91] *Ann Oncol*, 2019
MD Anderson	16	—	—	1/16 (6)	3/16 (18.8)	1.8	17.1	Negrao *et al.*[92] ASCO 2018
French Lung Cancer Group (GFPC)[b]	23	Nivolumab 83% ≥2 lines 100%	17%[c]	6/23 (27.3)	11/23 (50)	2.2 (1.7-15.2)	20.4 (9.3-NR)	Guisier *et al.*[93] *JTO*, 2020

CI, confidence interval; DCR, disease control rate; ICIs, immune checkpoint inhibitors; MSKCC, Memorial Sloan Kettering Cancer Centre; NR, not reached; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.
[a] Multicentre study of 551 patients in 24 centres from 10 countries. The molecular alterations involved were *KRAS* ($n = 271$), *EGFR* ($n = 125$), *BRAF* ($n = 43$), *MET* ($n = 36$), *HER2* ($n = 29$), *ALK* ($n = 23$), *RET* ($n = 16$), *ROS1* ($n = 7$) and multiple drivers ($n = 1$).
[b] Multicentre study of 107 patients in 21 centres from France. In this case, the molecular alterations were *BRAF* ($n = 44$), *MET* ($n = 30$), *HER2* ($n = 23$) and *RET* ($n = 9$).
[c] Programmed death-ligand 1status unknown in 65% of patients.

The mechanisms of action of ADCs remain poorly understood and require additional translational studies with pre- and on-treatment biopsies to elucidate the unanswered speculations about these molecules and to better define the target population.

The internalization and ubiquitination of an ADC are mostly dependent on the presence of an *HER2* mutation or amplification rather than that of a simple HER2 overexpression, which could explain the higher activity of ADCs in the first two subgroups of HER2 molecular alterations.[81] Strategies that increase the dynamics of internalization may increase the efficacy of these drugs. In this context, Li *et al*. showed that the combination of T-DM1 with a pan-HER irreversible inhibitor, such as neratinib, enhanced receptor ubiquitination and subsequent internalization of HER2—ADC complexes, thus resulting in a potent antitumour activity. They also demonstrated, both *in vitro* and *in vivo*, that ADC switching to T-Dxd, with its different cytotoxic payload, achieved durable responses after developing resistance to T-DM1.[81] Similar findings were reported by Robichaux *et al.* after testing low doses of poziotinib with T-DM1 in an *HER2*-mutant Y772dupYVMA NSCLC PDX model.[45]

Moreover, it has been shown that T-Dxd increases tumour-infiltrating CD8+ T cells and enhances the expression of PD-L1 by the major histocompatibility complex class I in breast cancer cells.[94] This is indeed the rationale behind combining T-Dxd with ICIs in patients with HER2-altered NSCLC, such as with durvalumab in the ongoing phase II HUDSON umbrella study of patients with NSCLC who have progressed on an anti-programmed cell death protein 1 (PD-1)/PD-L1 containing therapy (NCT03334617). Furthermore, DESTINY-Lung03 is a phase Ib trial that will investigate the safety, tolerability and efficacy of T-Dxd in combination with durvalumab and chemotherapy as a first-line treatment in patients with HER2-positive advanced NSCLC (NCT04686305). Additionally, another phase Ib trial will evaluate T-Dxd in combination with pembrolizumab in HER2-positive and *HER2*-mutant NSCLC patients who have not received prior treatment with anti-PD-1/PD-L1 or HER2 agents (NCT04042701).

CONCLUSION

New horizons are being explored with the advent of HER2-targeted therapies in HER2-altered advanced NSCLC, thus bringing hope to this incurable disease. Although poziotinib and pyrotinib have shown greater activity against *HER2*-mutant NSCLC when compared to other TKIs, ADC-based therapies seem to offer the highest response rates and the best survival outcomes both in patients with *HER2*-mutant and HER2-positive refractory NSCLC patients. Furthermore, combination therapies are being investigated in order to enhance the efficacy of anti-HER2 agents. These new data reinforce the need to make HER2 testing a systematic reflex upon diagnosis of advanced NSCLC.

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