First-line immunotherapy or angiogenesis inhibitor plus chemotherapy for *HER2*-altered NSCLC: a retrospective real-world POLISH study

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Abstract

Background: There have been no comprehensive large-scale studies that have evaluated the benefits of chemotherapy-based regimens in addressing *HER2*-altered advanced non-small-cell lung cancer (NSCLC) in a first-line setting. Data on *HER2* alteration subtypes and concomitant alterations are also limited. Accordingly, our retrospective, real-world POLISH study assesses the efficacy of first-line chemotherapy alone (C) as well as combinations with immune checkpoint inhibitors (C + I) or angiogenesis inhibitors (C + A) for *HER2*-altered NSCLC; molecular features are also reported.

Methods: *HER2*-altered NSCLC patients who received a first-line treatment between November 2015 and September 2021 were screened. Patients treated with C, C + I, or C + A were included in our final efficacy analysis. Progression-free survival (PFS) was compared between the subgroups. A Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was performed to evaluate concomitant alterations.

Results: A total of 293 patients were screened, with an identification of *HER2* amplification and 37 distinct *HER2* mutations, and 210 cases treated with C, C + I, or C + A were ultimately included. C + A achieved longer PFS than C (5.63 *vs* 4.03 months, hazard ratio: 0.64, 95% confidence interval [CI]: 0.46–0.88, *p* = 0.006). C + I did not improve median PFS compared to C + A or C (both *p* > 0.05), despite the programmed cell death ligand-1 (PD-L1) expression or tumor mutational burden. KEGG analysis revealed that concomitant upregulation of *PI3 K/AKT* pathway signaling was common in *HER2*-altered NSCLC.

Conclusion: Chemotherapy plus angiogenesis inhibitors may yield a greater survival benefit than chemotherapy alone in a first-line setting for *HER2*-altered NSCLC, whereas an immune-based combination therapy may not be superior to a sole chemotherapy regimen. Activation of *PI3 K/AKT* signaling may mediate immunosuppression in *HER2*-altered NSCLC.

Keywords: angiogenesis inhibitor, chemotherapy, *HER2* alteration, immunotherapy, non-small-cell lung cancer, real-world study

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Introduction

Lung cancer remains the most fatal malignant disease worldwide, with a 5-year relative survival rate of 5% when metastasis is diagnosed.[1] Recent approval of various targeted agents have greatly benefited the prognoses of patients carrying driver genes; these include epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), and c-ros oncogene 1 receptor tyrosine kinase (*ROS-1*). Further research into pathogenic mechanisms has revealed other oncogenic drivers in lung cancer, such as human epidermal growth factor receptor 2 (*HER2*, also known as *ERBB2*).[2]

As member of the *ERBB* receptor tyrosine kinase family, *HER2* presents three primary oncogenic-activation mechanisms: *HER2* mutation, amplification, and protein overexpression.[3] In non-small-cell lung cancer (NSCLC), *HER2* mutations and amplification are uncommon, accounting for only 1.6–3% and 2–4%, respectively. The most common mutational subtype is A775_G776insYVMA, a 12-base pair (bp) in-frame insertion in exon 20.[4–6] Numerous studies have demonstrated that post-chemotherapy prognoses of NSCLC patients with *HER2* alterations are unfavorable, with a median progression-free survival (PFS) of 4.9–5.9 months and median overall survival (OS) of 9.9–10.7 months in first-line or second-line settings.[7,8]

In the past five years, immune checkpoint inhibitors (ICIs) of anti-programmed cell death protein 1 (PD-1) or programmed cell death ligand 1 (PD-L1) antibodies have emerged as a standard first-line treatment for oncogene-naïve advanced NSCLC, as it has demonstrated greater benefits than chemotherapy. Nevertheless, several large randomized clinical trials have linked oncogenic drivers such as *EGFR* and *ALK* with poor responses to immunotherapy.[9,10] Currently, there is no available comprehensive study evaluating the efficacy of first-line combination strategies using chemotherapy plus angiogenesis inhibitors or ICIs for advanced NSCLC patients with *HER2* alterations. Little is known about how these therapies interact with the heterogeneous landscape of *HER2* alterations in NSCLC, or why these patients have a poor response to ICIs. Therefore, we conducted this large cohort real-world study to investigate the clinical outcomes of chemotherapy alone, chemotherapy plus ICIs, or angiogenesis inhibitors in advanced NSCLC patients with *HER2* alterations in a first-line setting. We also performed a molecular analysis to explore the possible mechanisms underlying the minimal response to ICIs in this subset population.

Patients and methods

Study design and population
Data of metastatic NSCLC patients harboring *HER2* alterations who received first-line systemic therapies at the National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences between November 30, 2015, and September 1, 2021, were retrospectively reviewed. The inclusion criteria were as follows: (1) ⩾18 years of age,

(2) histologically or cytologically confirmed metastatic NSCLC, (3) confirmed *HER2* mutations or *HER2* amplification (defined as *HER2* copy number ⩾3.62[11]) at primary diagnosis detected *via* next-generation sequencing (NGS) testing with tumor tissues or liquid biopsy samples, and (4) pretreatment with first-line therapies. In addition, patients treated with *HER2*-targeted therapy, an angiogenesis inhibitor or ICI monotherapy, or an angiogenesis inhibitor plus ICI combined with or without chemotherapy, as well as those without available data for efficacy evaluation, were excluded from the efficacy analysis. PD-L1 expressions were detected using a Dako 22 C3 pharmDx test kit. The PD-L1 tumor proportion score (TPS) was calculated as the percentage of ⩾100 viable tumor cells with complete or partial membrane staining. Tumor mutational burden (TMB), defined as the number of somatic, coding base substitutions, and short insertions and deletions per megabase of the genome examined, was assessed using formalin-fixed, paraffin-embedded tissue samples or blood using the NGS method covering various gene panels. NGS testing was performed in institutional laboratories or qualified third-party genetic testing companies that had acquired the national quality system certification, and all NGS testing was performed based on the Illumina sequencing system. Lesions were identified according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. All clinical data were extracted from electronic records. Informed consent was exempted from this retrospective observational study. The study was approved by the Ethics Committee of the National Cancer Center (approval number: 18-070/1648) and conducted in accordance with the Declaration of Helsinki.

Treatment and assessment
Patients who met the above inclusion criteria were included in the *HER2* molecular analysis (HMA) set, and those who received first-line chemotherapy alone (C), chemotherapy plus angiogenesis inhibitors (C + A), or chemotherapy plus ICIs (C + I) were included in the *HER2* efficacy analysis (HEA) set. Patients received chemotherapy, ICIs, and/or angiogenesis inhibitors at a standard dose according to the guidelines in clinical practice. Baseline images of measurable target lesions were obtained with computed tomography of the chest and abdomen and magnetic resonance imaging of the brain. Guidelines from the RECIST version 1.1 were used to classify a

complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). PFS was defined as the duration from the initiation of first-line therapy to the date of documented disease progression by the investigator or death from any cause. The objective response rate (ORR) was defined as the percentage of confirmed CR and PR. The disease control rate (DCR) was defined as the percentage of patients with CR, PR, and SD. OS was defined as the time from the initiation of first-line therapy to death. Both current and former smokers were classified as 'smokers', whereas non-smokers were those who smoked fewer than 100 cigarettes in their lifetime.

Statistical analysis

Statistical analyses were conducted using SPSS (version 23.0, SPSS Inc., Chicago, IL, USA). Patients in the HEA set were divided into three subgroups according to their first-line regimen. Continuous variables were summarized using medians and ranges, and categorical variables were described using frequency and percentage. Comparisons among the subgroups were accordingly performed using analysis of variance or the chi-square test. PFS and OS were analyzed using the Kaplan–Meier method. PFS values between different subgroups were compared using the log-rank test (two-sided), and the corresponding hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using the Cox proportional regression model. $P < 0.05$ was considered statistically significant. The p values for these analyses are nominal, and all are two-sided. Survival curves were plotted using GraphPad Prism version 5.0. A Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed in R version 4.0.5 with DAVID version 6.8 to reveal differentially expressed concomitant mutations with *HER2* alterations.

Results

Patient characteristics

In total, 293 metastatic NSCLC patients harboring *HER2* mutations or *HER2* amplification were included in the HMA set. All patients had adenocarcinoma, and no eligible squamous disease was found. As shown in the flow chart (Supplementary Figure 1), 76 patients received first-line *HER2*-targeted therapy, two patients received angiogenesis inhibitors alone, and four received PD-1 inhibitors alone or plus angiogenesis inhibitors. Except for one patient with no available data for the efficacy evaluation owing to a loss from follow-up after the first cycle of C + A, the remaining 210 patients in the HEA set were all treated with first-line chemotherapy-based regimens, including C ($n = 83$, 39.5%), C + A ($n = 81$, 38.6%), and C + I ($n = 46$, 21.9%). In the HEA set, the PD-L1 expression was observed in 71 patients (33.8%). Thirty-seven patients (17.6%) had a TPS of <1%. Twenty-three patients (11.0%) had $1 \leqslant TPS < 50\%$, and 11 patients (5.2%) had TPS < 50%. In addition, 48 cases (22.9%) were available to assess the TMB values. Thirty-two cases (15.2%) had TMB values of less than 10 Muts/Mb, and only 6 cases (2.9%) were greater than 10. There was no difference in baseline characteristics among the subgroups, except for the PD-L1 expression ($p = 0.01$; Table 1).

Molecular landscape of HER2 alterations

The *HER2* mutation or amplification statuses were determined using tumor tissues in 194 (92.4%) patients and from plasma in 16 (7.6%) patients. In the HMA set, apart from the *HER2* amplification ($n = 12$, 4.0%), NGS testing identified 37 distinct *HER2* mutations, including 16 types of insertions, 19 types of missense mutations ($n = 34$, 11.6%), and 2 types of deletions (Figure 1). The most common alteration subtype was the exon 20 insertion (ex20ins, $n = 246$, 83.9%); the 12-bp in-frame insertion A775_G776insYVMA ($n = 166$, 56.7%) was most dominant, followed by the 6-bp insertion G776delinsVC ($n = 30$, 10.2%), 9-bp insertion P780_Y781insGSP ($n = 29$, 9.8%), and other ex20ins variants ($n = 21$, 7.2%). In addition, three patients were identified with concomitant ex20ins and missense mutations, including G778dup with G776 S, P780_Y781insGSP with L785 F, and A775_G776insYVMA with S783A. The other three cases were deemed A775_G776insYVMA with *HER2* amplification.

KEGG analysis for concomitant alterations

The Venn plot demonstrates the top-10 concomitant genes together with *HER2* detected by NGS testing among HEA-set patients at primary diagnosis ($n = 101$, 48.1%); *TP53* ($n = 71$, 70.3%) aberrations were the most frequent co-mutations observed in this study (Figure 2(a)). In addition, 27 cases (26.7%) presented concomitant alterations in the cell cycle pathway signaling, including
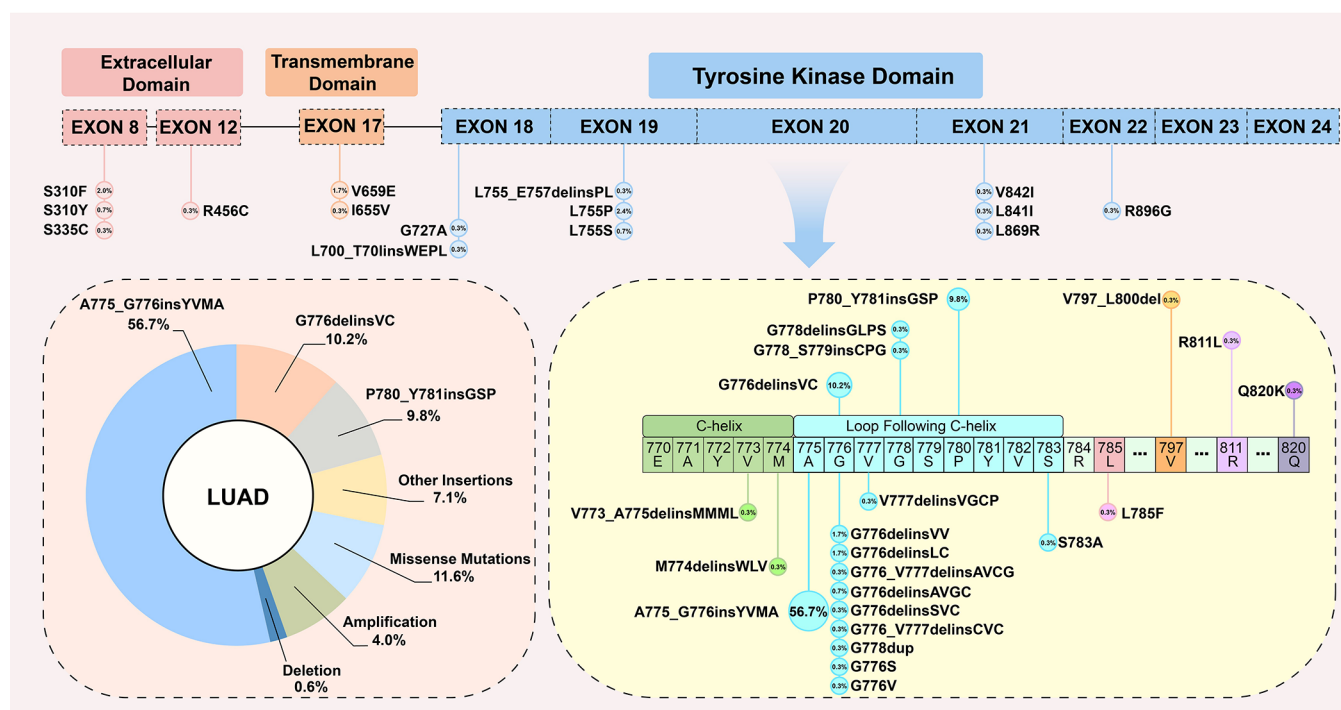
Figure 1. Proportion and detailed molecular landscape of NSCLC patients with distinct *HER2*-activating alterations.
LUAD, lung adenocarcinoma.

CDKN2A, *MDM2*, *MDM4*, *CDK4*, *CDK12*, *CCNE1*, *CCND1*, and *RB1*. Fourteen patients (13.9%) had concomitant alterations in the *PI3 K*/*AKT* signaling pathway, including *AKT1*, *AKT2*, *PIK3CA*, and *PTEN*. KEGG analysis revealed differentially expressed concomitant alterations in patients with *HER2* heterogeneous mutations or amplification. KEGG analysis was performed for the 15 significant signaling pathways. Among these, pathways in cancer, *PI3 K*/*AKT*, and the cell cycle comprised the majority, at 39, 25, and 26 counts, respectively. In addition, other concomitant signaling pathways that might mediate drug resistance in *HER2*-altered NSCLC have also been identified and reported, including microRNAs in cancer, *MAPK*, *HIF-1*, and *ERBB* signaling pathways (Figure 2(b)).

Regimens and efficacy

Among the 210 patients available for first-line treatment efficacy analysis, 187 had PFS events. Eighty-three patients (39.5%) received C, and the majority (*n* = 71, 85.5%) were treated with platinum/pemetrexed. The ORR, DCR, median PFS, and median OS of C were 16.9% (14/83), 89.2% (74/83), 4.03 months (95% CI: 2.70–5.37), and 31.67 months (95% CI: 29.63–33.71),

respectively. Eighty-one patients (38.6%) were treated with C + A, and most received platinum/pemetrexed plus bevacizumab (*n* = 69, 85.2%). The ORR, DCR, median PFS, and median OS of C + A were 23.8% (19/80), 91.3% (73/80), 5.63 months (95% CI: 4.84–6.43), and 36.27 months (95% CI: 28.71–42.83), respectively. Forty-six patients (21.9%) were administered C + I, and nearly all (*n* = 45, 97.8%) received platinum/pemetrexed chemotherapy plus PD-1 inhibitors. One patient received nedaplatin/pemetrexed plus the PD-L1 inhibitor atezolizumab. The ORR, DCR, and median PFS of C + I were 28.9% (13/45), 80.0% (36/45), and 5.20 months (95% CI: 3.64–6.76), respectively. At the cutoff time, the median OS for C + I was immature because only two deaths had occurred. The 6-month and 12-month survival rates of C + I were 75.6% (34/45) and 53.3% (24/45), respectively. The PFS based on the above three treatment patterns is listed in Figure 3(a).

Significant PFS differences were observed between subgroups C + A and C (median, 5.63 *vs* 4.03 months, HR = 0.64, 95% CI: 0.46–0.88, *p* = 0.006). No difference in PFS was observed between subgroups C + I and C (median, 5.20 *vs* 4.03 months, HR = 0.77, 95% CI: 0.52–1.14,

Table 1. Clinicopathological characteristics of *HER2*-altered patients in first-line treatment.

Characteristics	Total (*n* = 210)	C (*n* = 83)	C + A (*n* = 81)	C + I (*n* = 46)	*p* value
Age (years)	54.7 ± 9.92	54.6 ± 8.94	54.4 ± 10.82	55.4 ± 10.13	0.61
Gender					0.51
Female	112 (53.3%)	46 (55.4%)	45 (55.6%)	21 (45.7%)	
Male	98 (46.7%)	37 (44.6%)	36 (44.4%)	25 (54.3%)	
Smoking history					0.88
Never	139 (66.2%)	56 (67.5%)	54 (66.7%)	29 (63.0%)	
Current/former	71 (33.8%)	27 (32.5%)	27 (33.3%)	17 (37.0%)	
CNS metastases					0.61
Presence	28 (13.3%)	11 (13.3%)	9 (11.1%)	8 (17.4%)	
Absence	182 (86.7%)	72 (86.7%)	72 (88.9%)	38 (82.6%)	
NGS specimen					0.80
Tumor tissue	194 (92.4%)	78 (94.0%)	74 (91.4%)	42 (91.3%)	
Plasma	16 (7.6%)	5 (6.0%)	7 (8.6%)	4 (8.7%)	
HER2 variants					0.50
Ex20ins	178 (84.8%)	72 (86.7%)	70 (86.4%)	36 (78.3%)	
Missense	20[a] (9.5%)	6 (7.3%)	8 (9.7%)	6 (13.0%)	
Amplification	13[b] (6.2%)	5 (6.0%)	3 (3.7%)	5 (10.9%)	
Deletion	1 (0.5%)	0	1 (1.2%)	0	
PD-L1 expression					0.01
Negative	37 (17.6%)	11 (13.3%)	21 (25.9%)	5 (10.9%)	
1 ≤ TPS < 50%	23 (11.0%)	5 (6.0%)	7 (8.6%)	11 (23.9%)	
TPS ≥ 50%	11 (5.2%)	3 (3.6%)	5 (6.2%)	3 (6.5%)	
NA	139 (66.2%)	64 (77.1%)	48 (59.3%)	27 (58.7%)	
TMB value (Mb/Muts)					0.30
<10	32 (15.2%)	9 (10.8%)	17 (21.0%)	6 (13.0%)	
≥10	6 (2.9%)	4 (4.8%)	1 (1.2%)	1 (2.2%)	
NA	172 (81.9%)	70 (84.4%)	63 (77.8%)	39 (84.8%)	
Chemotherapy regimens					0.13
Platinum/pemetrexed	185 (88.1%)	70 (84.3%)	71 (87.7%)	44 (95.7%)	
Other platinum-based regimens	25 (11.9%)	13 (15.7%)	10 (12.3%)	2 (4.3%)	

C, chemotherapy alone; C + A, chemotherapy plus angiogenesis inhibitors; C + I, chemotherapy plus immune checkpoint inhibitors; Ex20ins, exon 20 insertion; NA, not available; TMB, tumor mutational burden; TPS, tumor proportion score.
[a]One patient was detected to be exon 20 insertion G778dup with G776 S missense mutation.
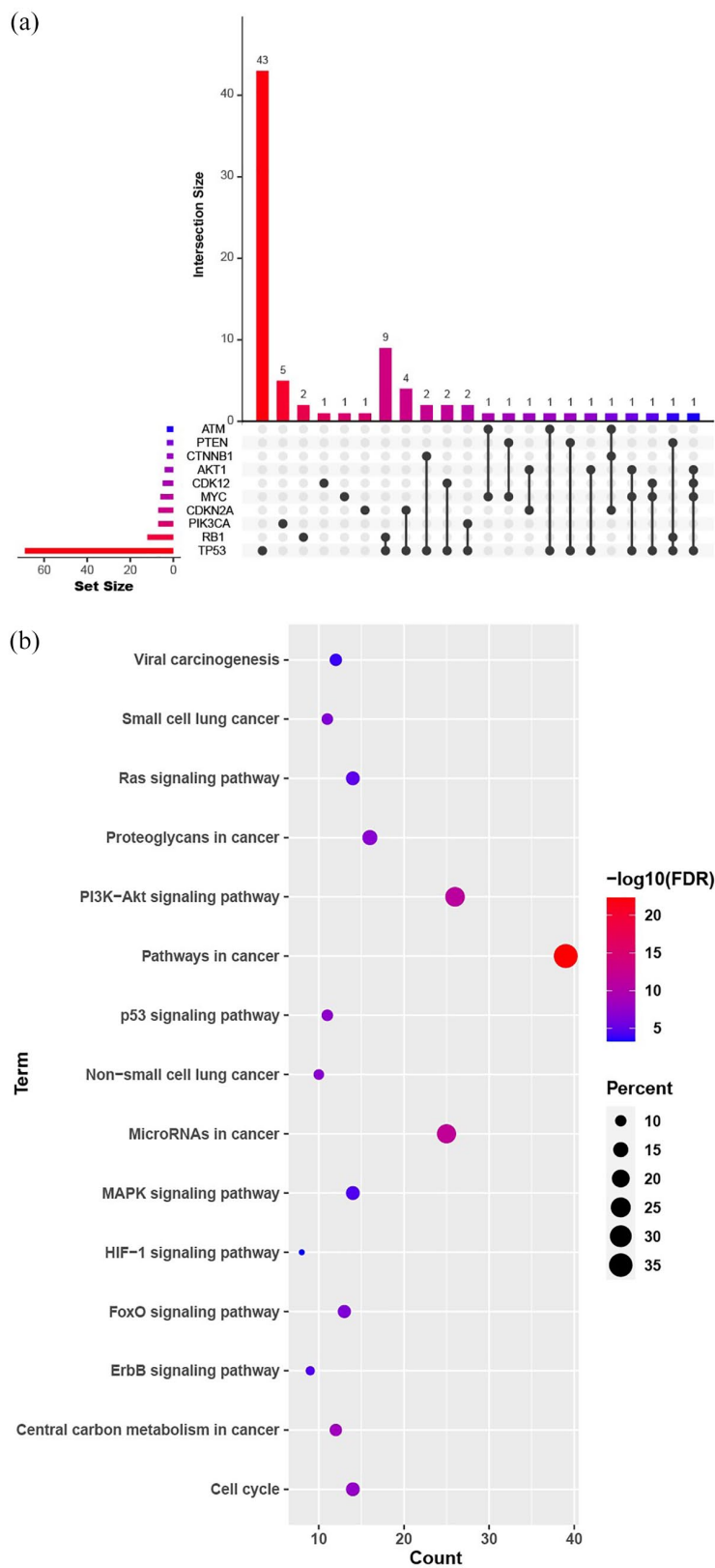[b]One patient was detected to be exon 20 insertion A775_G776insYVMA with *HER2* amplification.

Figure 2. (a) Top 10 concomitant altered genes together in NSCLC patients with *HER2* alterations detected using next-generation sequencing among patients at primary diagnosis as per Venn plot and (b) Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of concomitant signaling pathways in patients with *HER2* mutations and *HER2* amplification.

$p = 0.20$), or C + A and C + I (median, 5.63 *vs* 5.20 months, HR = 0.84, 95% CI: 0.56–1.25, $p = 0.37$). There was no difference in OS between C + A and C (median, 36.27 *vs* 31.67 months, HR = 1.07, 95% CI: 0.64–1.78, $p = 0.80$). Similarly, no statistical differences in PFS were observed between *HER2* alterations (amplification *vs* ex20ins mutations *vs* missense mutations) and between patients with or without brain metastases (Supplementary Figures 2 and 3).

PD-L1 and TMB as biomarkers for ICI efficacy evaluation

Among the 45 patients receiving C + I and available for efficacy evaluation, 19 had molecular data for PD-L1 expression. For five patients with TPS negativity, the ORR was 20% (1/5), and the median PFS was 7.67 months (95% CI: 0.28–15.1). Among the 11 patients who had $1 \leqslant$ TPS $< 50\%$, ORR was 18.2% (2/11), with a median PFS of 2.80 months (95% CI: 0.87–4.73). Of note, for three patients with TPS $\geqslant 50\%$ (two with TPS 50%, and one with TPS 95%), only one achieved PR, with a PFS of 2.33 months. One patient achieved SD with a PFS of 7.90 months, and the other developed PD at the initial response evaluation, with a PFS of 1.27 months (Figure 3(b)).

Among seven patients who had detected TMB per NGS, one with a TMB of 12.7 Muts/Mb showed PD to PD-1 inhibitors with pemetrexed chemotherapy, with a short PFS of 1.27 months. For two patients who responded to PR and with a PFS of 5.97 and 7.67 months, their TMB values were 0 and 4.8 Muts/Mb, respectively.

Discussion

Our study provides comprehensive real-world evidence regarding the clinical efficacy of various therapies for advanced NSCLC with heterogeneous *HER2* alterations in a first-line setting. We examined full-scale heterogeneous genetic alterations of *HER2*, including 16 types of insertions, 19 types of missense mutations, 2 types of deletions and amplification. In accordance with previous studies, ex20ins was the most prevalent variant, and the predominant variant was A775_G776insYVMA, followed by G776delinsVC and P780_Y781insGSP.[12,13]

In this study, the overall median PFS values of the first-line treatments were 4.03 months for C, 5.63 months for C + A, and 5.20 months for

C + I, respectively. These outcomes are similar to previous studies that have indicated a PFS of 1.9–7.5 months for *HER2*-mutant NSCLC.[8,13–23] The outcomes of chemotherapy-based regimens and PD-1/PD-L1 inhibitors alone as a first-line treatment for advanced NSCLC with *HER2* alterations in a real-life setting are listed in Table 2. Notably, ours is one of the few studies to analyze the clinical outcomes of chemotherapy in combination with angiogenesis inhibitors for NSCLC carrying *HER2* alterations. Additional angiogenesis inhibitors can enhance drug delivery to tumor sites *via* 'vessel normalization'.[24] Thus, a synergistic effect might result in increased anti-tumor activity. Recently, Offin *et al.*[25] reported that *HER2*-mutated NSCLCs were associated with an increased risk of brain metastases development, and that *HER2*-mutant patients with brain metastases had a worse prognosis than those without brain metastases. In this study, 13.3% of patients had brain metastases at the baseline, and their mPFS was 3.97 months; these results are numerically inferior to those without brain metastases (5.03 months, HR: 0.80, 95% CI: 0.53–1.21).

In the pre-immunotherapy era, *HER2* amplification was considered as both predictive in NSCLC patients treated with gefitinib as well as a mechanism of resistance to *EGFR* tyrosine kinase inhibitors (such as afatinib and lapatinib) in 12% of non-T790M mutant NSCLC patients.[26,27] In recent years, immunotherapy has revolutionized the therapeutic paradigms for advanced NSCLC patients. However, increasing evidence has shown that *HER2*-altered NSCLC patients appear to be insensitive to ICIs.[28] Similarly, our study has also demonstrated that ICIs in combination with chemotherapy might not improve survival outcomes over chemotherapy alone in patients with *HER2*-altered disease, including *HER2* amplification. However, these results should be interpreted with caution owing to a small sample size. In addition, PD-L1 expressions and TMB values have reportedly impacted immunotherapy efficacy in many clinical trials.[29,30] In this study, we also analyzed this relationship. Unfortunately, patients with high PD-L1 expression and TMB did not show a significant improvement in PFS compared to other patients. Our observation was consistent with another study that demonstrated no obvious difference in PFS between *HER2*-mutant NSCLC with different PD-L1 TPS.[22]

Preclinical data showed that *HER2* amplification may abolish phosphorylation of *TANK*-binding
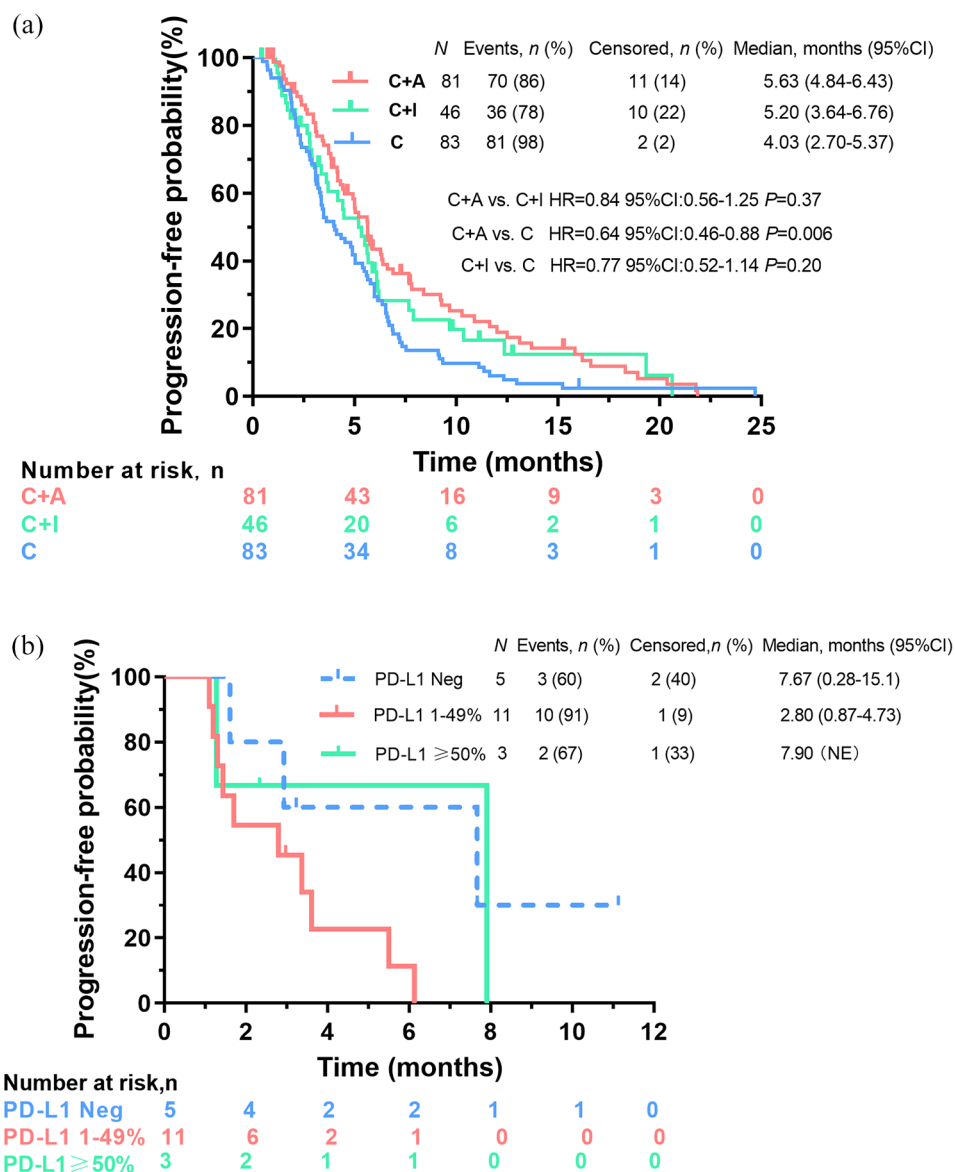
(a)

	N	Events, n (%)	Censored, n (%)	Median, months (95%CI)
C+A	81	70 (86)	11 (14)	5.63 (4.84-6.43)
C+I	46	36 (78)	10 (22)	5.20 (3.64-6.76)
C	83	81 (98)	2 (2)	4.03 (2.70-5.37)

C+A vs. C+I HR=0.84 95%CI:0.56-1.25 *P*=0.37

C+A vs. C HR=0.64 95%CI:0.46-0.88 *P*=0.006

C+I vs. C HR=0.77 95%CI:0.52-1.14 *P*=0.20

Number at risk, n

C+A	81	43	16	9	3	0
C+I	46	20	6	2	1	0
C	83	34	8	3	1	0

(b)

	N	Events, n (%)	Censored, n (%)	Median, months (95%CI)
PD-L1 Neg	5	3 (60)	2 (40)	7.67 (0.28-15.1)
PD-L1 1-49%	11	10 (91)	1 (9)	2.80 (0.87-4.73)
PD-L1 ≥50%	3	2 (67)	1 (33)	7.90 (NE)

Number at risk, n

PD-L1 Neg	5	4	2	2	1	1	0
PD-L1 1-49%	11	6	2	1	0	0	0
PD-L1 ≥50%	3	2	1	1	0	0	0

Figure 3. (a) Kaplan–Meier curves of PFS in patients harboring *HER2*-activating alterations with chemotherapy alone (C) *vs* chemotherapy plus angiogenesis inhibitors (C + A) *vs* chemotherapy plus ICIs (C + I) in a first-line setting and (b) PFS outcomes among subgroups with different programmed cell death ligand 1 (PD-L1) tumor proportion score.
HR, hazard ratio.

kinase 1 (*TBK1*) and attenuate stimulator of interferon genes (*STING*) signaling, thus suppressing type-I and -II interferon and antitumor immune responses.[31,32] Our KEGG analysis revealed that upregulation of *PI3 K/AKT* signaling was common in NSCLC patients with *HER2* mutations or amplification, which has also been reported in other *HER2*-positive solid tumors.[33–35] We supposed that the *PI3K/AKT* signaling, together with other signaling pathways related to the cell cycle and microRNA, might influence drug resistance

to ICIs in *HER2*-altered NSCLC. Regarding possible mechanisms explaining the immunosuppressive microenvironment in *HER2*-altered NSCLC, we propose that *HER2* mutations or amplification may activate the downstream *MAPK* and *PI3K/AKT* signaling pathways. Specifically, *AKT1* recruitment may impair *TBK1* phosphorylation and suppress interferon regulatory factor 3 (*IRF3*), thereby disrupting *STING* signaling in antitumor immune responses (Figure 4). Therefore, novel treatment strategies targeting immunosuppressive

Table 2. Reported retrospective studies on efficacy of first-line chemotherapy or ICIs for advanced NSCLC with *HER2* alterations.

Author	Year	Sample size[a]	Therapeutics	ORR (%)	DCR (%)	Median PFS (months)
Eng *et al.*[14]	2016	38	Chemotherapy	NA	NA	7.5
Mazières *et al.*[15]	2016	93 (101)	Chemotherapy	43.5	70.7	6
Song *et al.*[16]	2016	14 (21)	Platinum-based chemotherapy	NA	NA	4.6
Wang *et al.*[17]	2018	25 (29)	Pemetrexed-based chemotherapy	36	92	5.1
Auliac *et al.*[8]	2019	20 (23)	Platinum-based chemotherapy + bevacizumab	61.5	84.6	6.7
Mazieres *et al.*[18]	2019	29	ICIs: PD-1/PD-L1 inhibitors alone	7	37	2.5
Guisier *et al.*[19]	2020	23	ICIs: PD-1/PD-L1 inhibitors alone	27.3	50	2.2
Xu *et al.*[13]	2020	75	Chemotherapy	NA	NA	5.5
Chen *et al.*[20]	2021	21	ICIs: PD-1/PD-L1 inhibitors alone	0	NA	1.9
Lau *et al.*[21]	2021	14	ICIs: PD-1 inhibitors alone or plus CTLA-4 inhibitor	29	57	3.6
Saalfeld *et al.*[22]	2021	27 (61)	5 cases with PD-1 inhibitors alone 22 with PD-1 inhibitors + platinum-based chemotherapy	PD-1 inhibitors alone: 20 PD-1 inhibitors + platinum-based chemotherapy: 52	NA	PD-1 inhibitors + platinum-based chemotherapy: 6.0
Yang *et al.*[23]	2021	82 (98)	Chemotherapy	37.8	84.1	5.77

DCR, disease control rate; ICI, immune checkpoint inhibitor; NA, not available; ORR, overall response rate; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; PFS, progression-free survival.
[a]Expressed as number in the first-line setting (total number).

signaling pathways together with *HER2* should be developed to optimize targeted immunotherapy in this subset of patients.

In addition, we identified and reported other concomitant alterations in several signaling pathways that likely mediate drug resistance to anti-*HER2* therapeutics. *TP53* aberrations were the most common co-mutations in this study, thereby corroborating previous studies showing that concomitant *TP53* mutations were predominant among *HER2* ex20ins.[36,37] In addition, KEGG analyses may provide additional details on drug resistance in NSCLC patients with *HER2* heterogeneous alterations. Recent studies have found that microRNAs strongly influence chemotherapy resistance to cancer, including NSCLC.[38] The dysregulation of microRNAs is closely related to remodeling of tumors and the microenvironment.[39] In addition to the pathway mentioned above, other signaling pathways, including the cell cycle, *MAPK*, *HIF-1*, and *ERBB* were also reportedly involved in drug resistance in NSCLC.[40–43]

HER2-mutated NSCLCs have reportedly represented a distinct molecular subgroup of NSCLC.[44] Ninomiya *et al.*[45] reported that, compared to *EGFR*- and *ALK*-positive NSCLC, *HER2*-aberrant diseases were less responsive to targeted therapies. In addition, *HER2* ex20ins seems to have distinct characteristics from other *HER2* alterations (mainly non-ex20ins), with less sensitivity to conventional *HER2*-targeted TKIs (such as neratinib, afatinib, and dacomitinib), having only reached ORRs of 7.7–12% and median PFS of 2.9–5.5 months.[44] Recently, other novel *HER2*-targeted agents have been evaluated in *HER2*-altered NSCLC. A ZENITH20-2 study of poziotinib showed that the mPFS was 5.5 months and the ORR was 27% in patients with advanced *HER2* exon 20 mutant NSCLC.[46] Zhou *et al.*[12] reported a mPFS of 6.9 months and an ORR of 30% for pyrotinib as second-line or above therapy in patients with *HER2*-mutant advanced lung adenocarcinoma. In addition, ado-trastuzumab emtansine showed a mPFS of 5 months and an ORR of 44% for advanced NSCLC with *HER2* alterations.[47] Similarly, a DESTINY-Lung01
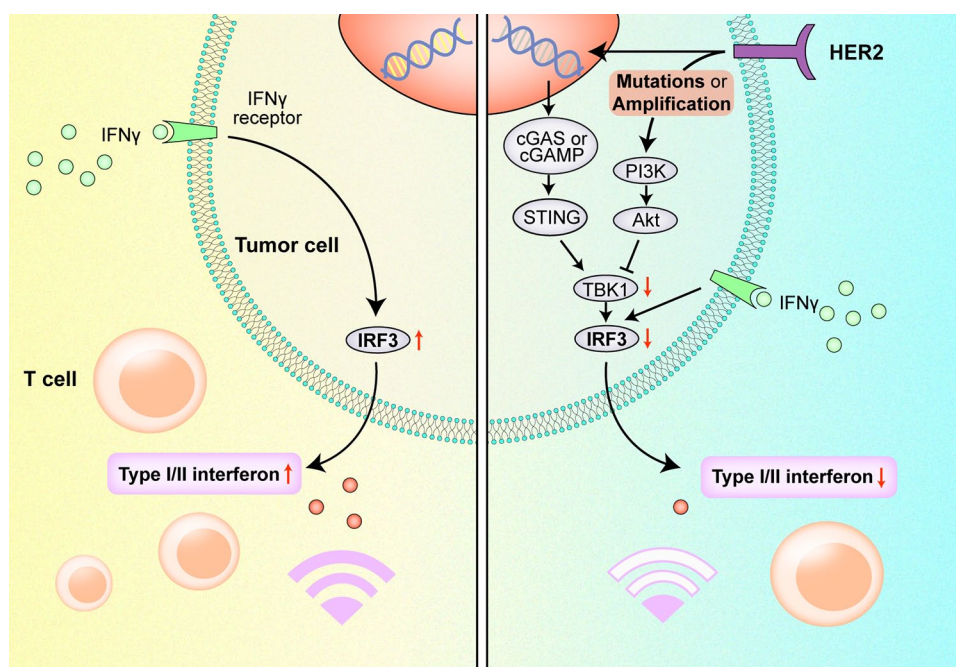
Figure 4. Possible mechanism for immunosuppression in *HER2*-altered NSCLC mediated by the *PI3 K/AKT* signaling pathway.

study reported a mPFS of 8.2 months and an ORR of 55% for trastuzumab deruxtecan in metastatic *HER2*-mutated NSCLC.[48] Although *HER2*-targeted therapies have greatly improved the prognoses of *HER2*-overexpressing breast cancer patients, they appear to be not ideal in patients with *HER2*-overexpressing NSCLC. A *HER2*-CS STUDY reported that patients with *HER2*-overexpressing NSCLC were insensitive to anti-*HER2* agents, with a response rate of 0%.[45] A *HER2* overexpressing cohort of DESTINY-Lung01 showed an ORR of 24.5% and an estimated mPFS of 5.4 months for trastuzumab deruxtecan.[49] However, the above studies on anti-*HER2* therapies were mainly focused on previously treated *HER2*-altered NSCLC. Further studies are needed to assess the effectiveness of anti-*HER2* agents in a first-line setting.

This study had several limitations. First, biases were inevitable owing to the retrospective nature of the study, and the treatment regimens were highly heterogeneous. In addition, although this study provided comprehensive *HER2* molecular subtypes and concomitant mutations in other signaling pathways, only half of the patients had available NGS data, and we did not have genetic information on other co-existing alterations or data on whole exome sequencing. Another limitation was the lack of *in vitro* evidence of tumor immunohistochemistry or flow cytometry data of the *PI3 K/AKT* pathway and other immunosuppressive genes. Finally, we included a relatively limited number of patients with *HER2* missense mutations and amplification, which prevented us from providing corroborative data on the subgroups.

In conclusion, this comparative POLISH study provides a novel insight into the outcomes of advanced *HER2*-altered NSCLC patients receiving a chemotherapy-based regimen as a first-line treatment in a real-world setting. First-line chemotherapy plus angiogenesis inhibitors might confer additional survival chances in patients with NSCLC with *HER2* alterations, as compared to using chemotherapy alone. The addition of ICIs to chemotherapy did not yield an improved survival benefit. Future prospective studies are required to verify these perspectives in first-line treatment settings for *HER2*-altered NSCLC.

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Data availability

Data supporting the results presented in this study are available from the corresponding author upon reasonable request.

Supplemental material

Supplemental material for this article is available online.

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