



Exon 20 YVMA insertion is associated with high incidence of brain metastasis and inferior outcome of chemotherapy in advanced non-small cell lung cancer patients with HER2 kinase domain mutations

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Background: Chemotherapy remains the standard care for *HER2* mutated advanced non-small cell lung cancer (NSCLC) even though several targeted drugs showed promising results in preliminary stages. This study aimed to investigate the association of mutation variants with clinical features and the efficacy of chemotherapy in patients with *HER2* mutated advanced NSCLC.

Methods: ARMS-PCR was used to identify *HER2* mutation in patients without common oncogenic alterations. Patients with detailed information were further enrolled for analysis of clinical features and efficacy of first line chemotherapy. Survival data was analyzed by Kaplan-Meier method and compared by log-rank test. Brain metastasis incidence was analyzed and compared by Gray's test.

Results: YVMA insertion accounted for the majority (68.4%, 67/98) of *HER2* mutation, and associated with significantly higher incidence of baseline extrathoracic metastasis ($P=0.009$), notably brain metastasis ($P=0.004$). Among 82 patients those received first line chemotherapy, YVMA insertion remarkably associated with inferior treatment outcomes, namely, a significantly shorter median progression free survival (PFS) and lower objective response rate (ORR) both in total patients (PFS: 5.2 *vs.* 7.7 m, $P=0.038$; ORR: 30.9% *vs.* 51.9%, $P=0.09$) and pemetrexed subgroup (PFS: 5.2 *vs.* 6.5 m, $P=0.022$; ORR: 31.8% *vs.* 60.0%, $P=0.054$). Multivariate analysis further established YVMA insertion as prognostic factor of worse PFS both for total patients (HR =1.578, 95% CI, 0.956–2.606) and patients received pemetrexed-based chemotherapy (HR =1.789, 95% CI, 1.013–3.160). In addition, YVMA insertion associated with higher incidence of lifetime brain metastasis ($P=0.002$) compared by Gray's test, with estimated 12-month brain metastasis incidence as 40.2% compared with 3.6% in the non-YVMA group.

Conclusions: YVMA insertion is associated with a higher incidence of brain metastasis, and inferior outcomes to chemotherapy than non-YVMA variants in patients with advanced NSCLC and *HER2* kinase domain mutations, which emphasized the unmet need of more potent anti-cancer therapies with high blood-brain barrier (BBB) penetration capacity for patients with YVMA insertion.

Keywords: *HER2* mutation; YVMA insertion; brain metastasis; chemotherapy; non-small cell lung cancer (NSCLC)

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Introduction

Targeted therapies have dramatically revolutionized treatment paradigm with higher response rates, longer progression-free survival (PFS) and less side effects compared with chemotherapy in advanced non-small cell lung cancer (NSCLC) patients harboring *EGFR/ALK/ROS1/BRAF* alterations (1-4). Rare mutations, such as *KRAS*, *RET*, and *MET* are also recommended for molecular detection in those patients without common oncogenic mutations with several drugs approved for treatments or in clinical trials (5-8).

HER2 kinase domain mutation has been increasingly acknowledged as a promising therapeutic target in advanced NSCLC with an incidence of 4.8% in patients without sensitizing *EGFR* mutations (9-11). Multiple HER2-targeted drugs showed promising treatment efficacy (12-16) such as neratinib (17), poziotinib (18) and pyrotinib (15,19) which showed an ORR of 53.3% and PFS of 6.4 months in *HER*-mutated NSCLC in our previous report. Antibody drug conjugates (ADC) like T-DM1 (20,21) and DS-8201a (22,23) have also shown great potency in HER2 mutated NSCLC, with T-DM1 having been recommended by the NCCN guideline (5). However, currently, targeted drugs have not yet been approved and chemotherapy remains the current standard of care in this setting (24,25).

Increasing evidences have demonstrated that even within the same oncogenic driver, clinical characteristics and treatment efficacy could be dramatically different. For example, patients with *EGFR* 19del have longer PFS and survival benefit compared with patients with *EGFR* L858R substitution (26,27). Different *ALK* variants and *BRAF* mutation classes have also been demonstrated to correlate with discrepant treatment outcomes, survival outcomes, and baseline clinical features (28-33). Furthermore, mutation variants also associated with different resistance mechanisms. For example, *EGFR* 19del associates with a higher incidence of T790M (34), and the *ALK* variant 3 with a higher incidence of G1202R (30). In addition, mutation variants also have been demonstrated to be related to different levels of tumor mutation burden (TMB) (35). Same has also been observed in HER2 mutated NSCLC, recently a study demonstrated that patients with different

HER2 mutations showed discrepant responses to afatinib, a pan-HER inhibitor (36). Collectively, these data suggest that identification of mutation variants could guide the proper choice of therapeutic decision. This study aimed to comprehensively investigate the distribution of HER2 kinase domain mutation variants and their association with clinical features and response to chemotherapy in patients with *HER2* mutated advanced NSCLC. We present the following article in accordance with the STROBE reporting checklist (available at <http://dx.doi.org/10.21037/tlcr-20-559>).

Methods

Patients population

Patients with advanced NSCLC and identified with *HER2* mutation in Tongji University School of Medicine Thoracic Cancer Institute from January 2015 to November 2018 were collected and reviewed. Detailed clinicopathological characteristics and therapeutic outcomes, such as age, gender, smoking status ("never smoker" was defined as patients with the smoking dose of fewer than 100 cigarettes in their lifetime), tumor histology, TNM stage (IASLC 8th edition), Eastern Cooperative Oncology Group (ECOG) performance status (PS), treatment regimens used, and the outcomes of treatments were collected. In addition, details of brain metastatic status were also collected if available, including the number of lesions, if they were symptomatic or not, and the treatment administrated to brain metastasis. All procedures performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Ethics Committee of Shanghai Pulmonary Hospital, Tongji University School of Medicine. Because of the retrospective nature of the research, the requirement for informed consent was waived.

Molecular testing

From January 2015 to November 2018, 2,788 patients of *EGFR/ALK/ROS1/KRAS/BRAF* wild-type were tested for *HER2* mutations. Molecular testing was performed with the *EGFR*, *BRAF V600*, and *KRAS* Mutations Detection Kit (Amoy Diagnostics, Xiamen, China) based on amplification

refractory mutation system (ARMS) method, and the AmoyDx EML4-ALK and ROS1 Fusions Detection Kit (Amoy Diagnostics, Xiamen, China) using reverse transcriptase polymerase chain reaction (RT-PCR) per the instructions of manufacturer, respectively. Details are given in our previous reports (11,26,37-39).

HER2 mutation testing was performed with the AmoyDx *HER2* Mutation Detection Kit which covered the mutations of exon 19 and exon20, including 13 different base changes encoding 7 different amino acid changes (Amoy Diagnostics, Xiamen, China) using the method of ARMS, following the instructions provided by the manufacturer. Mutation variant detail was identified with DNA sequencing using primers with the following sequences: 5'GCC ATG GCT GTG GTT TGT GAT AGG3' (forward) and 5'ATC CTA GCC CCT TGT GGA CAT AGG3' (reverse), as described in our previous report (11).

Efficacy evaluation of chemotherapy

Objective tumor responses were determined according to Response Evaluation Criteria in Solid Tumors (RECIST version 1.1). PFS was defined as the time interval from the date of treatment initiation to documented disease progression or death of any cause, whichever occurred first. Patients without disease progression or death were censored. The last date of follow-up was March 15, 2019, and the median follow-up time was 14.1 months. Time to brain metastasis was defined as the time interval from the date of diagnosis to initial documentation of brain metastasis.

Statistical analysis

Categorical data were analyzed with Chi-square test or Fisher's exact test. Numerical data were analyzed with the Student's *t*-test. The log-rank test was used to compare the survival times between different patient cohorts. Cox proportional hazards regression analysis, hazard ratios (HRs) and 95% confidential intervals (CIs) were performed and calculated to determine the survival difference. Statistical analyses were performed using the SPSS statistical software (version 20.0; IBM Corporation, Armonk, NY, USA). Gray's test (40) was used to estimate and compare the lifetime incidence of brain metastasis by R software (version 3.5.2; R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was considered for 2-sided P values of less than 0.05.

Results

Patients' characteristics

Among 2,788 patients without *EGFR/ALK/ROS1/KRAS/BRAF* alterations, 4.2% (116/2,788) patients were found to have *HER2* mutation using AMRS-PCR method. Among them, 18 patients were excluded from the analysis, including 14 without mutation variant detail due to insufficient tissue sample and 4 without baseline clinicopathological features (Figure 1). Finally, 98 patients were enrolled. Among them, 67 patients were found to harbor YVMA insertion (68.4%), 14 patients with GSP insertion (14.3%), 8 patients with G776>VC (8.2%), 4 patients with G776>LC (4.1%), 2 patients with G776C (2.0%), 2 patients with L755P (2.0%), and 1 patient with V777M (1.0%) (Figure 2). The median age of enrolled patients was 60 (range, 33–84) years old; 53 (54.1%) were male; 91 (92.9%) were with lung adenocarcinoma; 28 (28.6%) were current/former smokers, and 78 (79.6%) were initially diagnosed as stage IV (Table 1).

Since YVMA insertion accounted for the majority of *HER2* mutation, patients were then divided as YVMA group (n=67) and non-YVMA group (n=31). Patients harboring YVMA insertion were found more likely to have extrathoracic metastasis at baseline compared with those in non-YVMA group (40/67, 59.7% vs. 9/31, 29.0%, P=0.009). Among the most prevalent extrathoracic metastatic sites, YVMA group was found to have a significantly higher incidence of brain metastasis (P=0.004), but not of liver metastasis (P=0.053) and bone metastasis (P=0.112). No significant differences in age, gender, histology, ECOG-PS, family cancer history, baseline disease stage and smoking history were observed between the two groups (Table 1).

We also compared the baseline clinicopathological features between patients with *HER2* mutations and patients without common oncogenic alterations (randomly selected, n=200). Patients with *HER2* mutations displayed a significantly higher proportion of females, young patients, histology as adenocarcinoma and never-smokers compared with patients without common oncodrivers (Tables S1,S2).

Efficacy of first-line chemotherapy between different HER2 mutation variants

Among 98 patients, 82 (83.7%) received chemotherapy alone as first-line treatment, 6 (6.1%) received targeted therapies including 5 with afatinib and 1 with pyrotinib, 1 (1.0%) received immunotherapy using pembrolizumab, 3 (3.1%) received thoracic radiotherapy either alone or

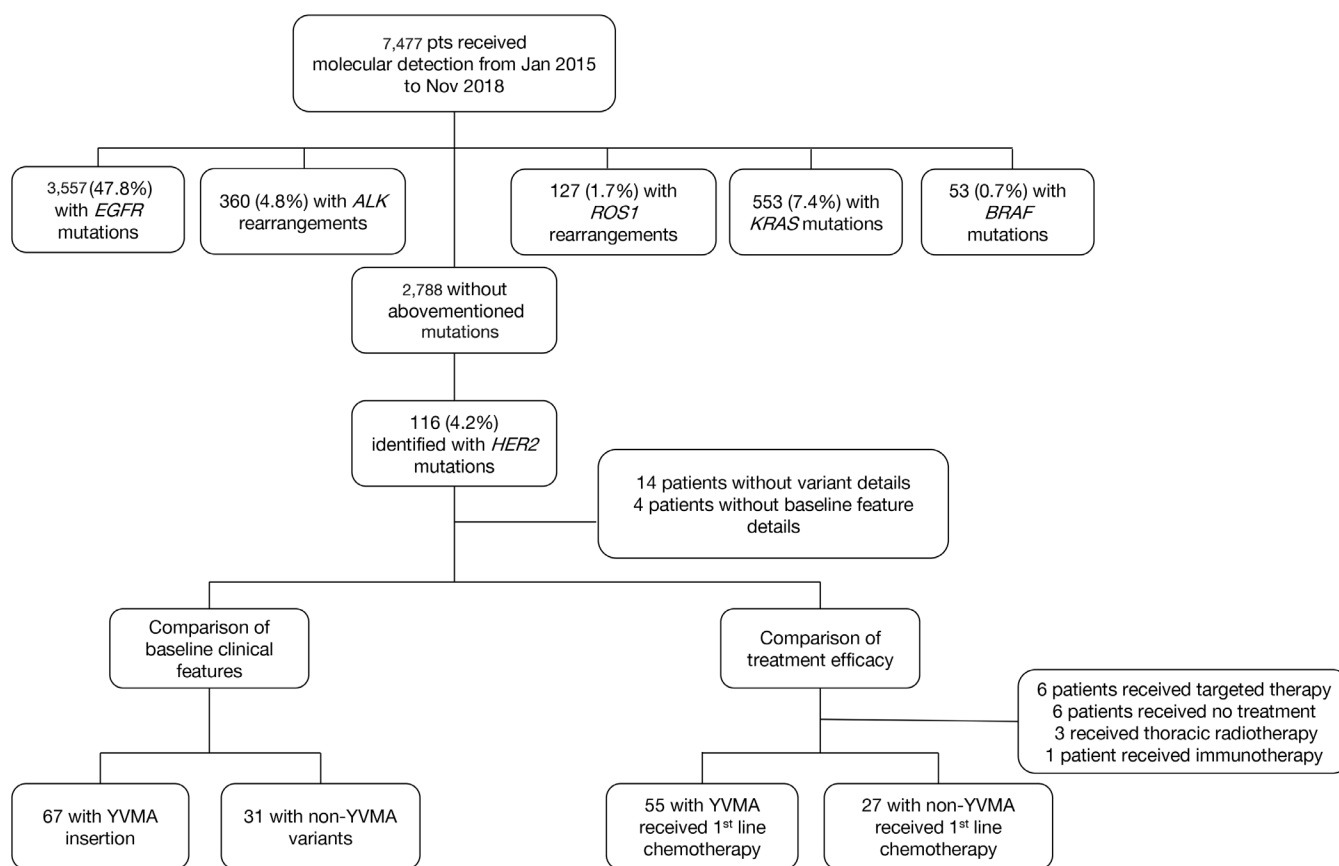


Figure 1 Flowchart of this study. A total of 98 patients were enrolled for comparison of the baseline clinicopathological features, and 82 patients were enrolled for comparison of the efficacy of first line chemotherapy.

combined with chemotherapy, and 6 (6.1%) received no anti-tumor treatment (treatment responses to targeted therapies are summarized in [Table S3](#)). Finally, 82 patients were enrolled for chemotherapy efficacy comparison. Among them, 55 (67.1%) were with YVMA insertion, and 27 (32.9%) with non-YVMA mutation variants. Concerning regimens used, the majority of patients (78.0%, 64/82) received pemetrexed-based chemotherapy (including 53 combined with platinum, 8 combined with platinum and bevacizumab, and 3 with pemetrexed alone, [Table S4](#)). No difference in the chemotherapy regimens was observed between two groups ($P=0.739$) ([Table 1](#)).

With a median follow-up time as 14.1 months, 73 patients experienced disease progression, while 9 patients were still on chemotherapy at last date of follow-up. The objective response rate (ORR) was 37.8%, and the disease control rate (DCR) was 84.1% ([Table 2](#)). Subgroup analysis showed that YVMA insertion ($n=55$) associated with inferior treatment outcomes compared with non-YVMA

variants ($n=27$) with lower ORR (30.9% *vs.* 51.9%, $P=0.09$), lower DCR (78.2% *vs.* 96.3%, $P=0.051$), and significantly shorter median PFS (median: 5.2 *vs.* 7.7 months, $P=0.038$) ([Table 2](#), [Figure 3](#)). Similarly, YVMA insertion ($n=40$) was also observed with inferior treatment outcomes, with lower ORR (31.8% *vs.* 60.0%, $P=0.054$), lower DCR (75.0% *vs.* 95.0%, $P=0.085$), and a significantly shorter median PFS (5.2 *vs.* 6.5 months, $P=0.022$) than non-YVMA variants ($n=24$) in patients received pemetrexed-based chemotherapy ($n=64$). In addition, we also compared the efficacy of chemotherapy between patients with *HER2* mutation ($n=82$) and patients without common oncogenic mutations (randomly selected, $n=50$), no significant differences in ORR (37.8% *vs.* 28%, $P=0.264$), DCR (84.1% *vs.* 82%, $P=0.812$) and median PFS (median: 5.8 *vs.* 5.1 months, $P=0.922$) were observed ([Table S2](#)).

Multivariate cox regression analysis, incorporating age, gender, histology, smoking history, ECOG PS, mutation variants group, and baseline disease involvement found

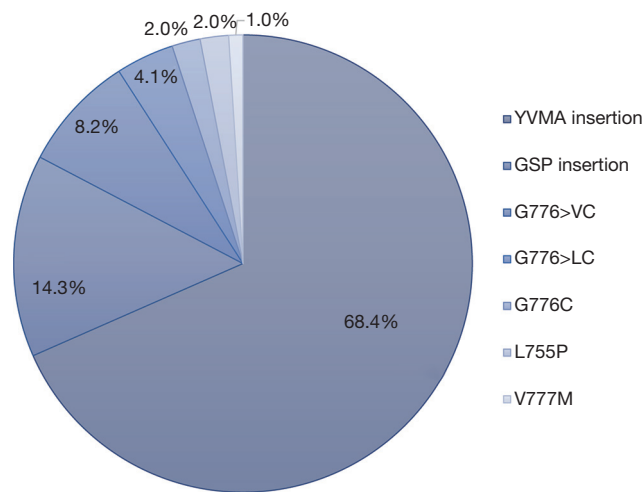


Figure 2 The spectrum of mutation variants of HER2 kinase domain of the enrolled 98 patients.

that patients with YVMA insertion associated with worse PFS compared with patients of non-YVMA variants both in total patients (HR =1.578, 95% CI, 0.956–2.606) and subgroup of pemetrexed-based chemotherapy (HR =1.789, 95% CI, 1.013–3.160) (Table 3). Since baseline extrathoracic metastases also associated with worse PFS in both the whole population and the pemetrexed subgroup, we then further performed multivariate analysis incorporating the most prevalent metastatic sites and found baseline bone metastases associated with worse PFS (HR =1.788, 95% CI, 1.072–2.981) and YVMA also associated with worse PFS (HR =1.633, 95% CI, 0.985–2.706). Similarly, subgroup analysis also showed YVMA insertion to be associated with worse PFS (HR =1.685, 95% CI, 0.931–3.051) in patients treated with pemetrexed-based regimens (Table S5).

Table 1 Baseline clinicopathological features and treatment regimens used in total patients and two groups.

Characteristics	Total n (%)	YVMA n (%)	Non-YVMA (N%)	P value
Number	98	67	31	
Age				
Median (range)	60 [33–84]	52 [33–84]	63 [34–74]	0.402
<65	71 (72.4)	49 (73.1)	22 (71.0)	1.0
Age ≥65	27 (27.6)	18 (26.9)	9 (29.0)	
Gender				0.828
Male	53 (54.1)	37 (55.2)	16 (51.6)	
Female	45 (45.9)	30 (44.8)	15 (48.4)	
Histology				0.840
Adenocarcinoma	91 (92.9)	62 (92.5)	29 (93.5)	
Squamous carcinoma	1 (1.0)	1 (1.5)	0	
NOS	5 (5.1)	3 (4.5)	2 (6.5)	
Adenosquamous carcinoma	1 (1.0)	1 (1.5)	0	
ECOG-PS				0.057
0	13 (13.3)	12 (17.9)	1 (3.2)	
1–2	85 (86.7)	55 (82.1)	30 (96.8)	
Smoking history				0.341
Ever smoker	70 (71.4)	50 (74.6)	20 (64.5)	
Never smoker	28 (28.6)	17 (25.4)	11 (35.5)	

Table 1 (continued)

Table 1 (continued)

Characteristics	Total n (%)	YVMA n (%)	Non-YVMA (N%)	P value
Stage of disease				0.925
III B/III C	12 (12.2)	8 (11.9)	4 (12.9)	
IV A/IV B	78 (79.6)	54 (80.6)	24 (77.4)	
Recurrent	8 (8.2)	5 (7.5)	3 (9.7)	
Disease involvement				0.009
Intrathoracic	49 (50.0)	27 (40.3)	22 (71.0)	
Extrathoracic	49 (50.0)	40 (59.7)	9 (29.0)	
Prevalent metastatic sites				
Brain	14 (14.3)	14 (20.9)	0	0.004
Liver	8 (8.2)	8 (11.9)	0	0.053
Bone	34 (34.7)	27 (40.3)	7 (22.6)	0.112
Treatment as first line				NE
Chemotherapy	82 (83.7)	55 (82.1)	27 (87.1)	
Targeted therapy	6 (6.1)	6 (9.0)	0	
Immunotherapy	1 (1.0)	1 (1.5)	0	
Unknown or without systemic treatment	9 (9.2)	5 (7.5)	4 (12.9)	
Chemotherapy regimens				0.739
Pemetrexed based	64 (78.0)	44 (80.0)	20 (74.1)	
Gemcitabine based	14 (17.1)	8 (14.5)	6 (22.2)	
Paclitaxel based	4 (4.9)	3 (5.5)	1 (3.7)	

NOS, not other specified; ECOG-PS, Eastern Cooperative Oncology Group Performance Score.

Correlation of HER2 mutation variants with the incidence of brain metastases

Since brain metastasis was found to be significantly much more prevalent in patients with YVMA insertion than in other patients, we then investigated the impact of mutation variants on the incidence of lifetime brain metastasis. Among 98 patients enrolled, 14 (14.3%) were found with baseline brain metastasis, and all of them were with YVMA insertion ($P=0.004$). The majority of patients (8/14, 57.1%) with baseline brain metastasis were with limited lesions (1-3) and asymptomatic brain metastasis (10/14, 71.4%). Among the four patients with symptomatic brain metastasis, three patients received cranial radiotherapy, including two with WBRT plus SRS and one with WBRT alone, and one patient received surgical resection of metastatic brain lesion

as initial treatment (detailed information of treatments used in patients with brain metastases were presented as [Table S6](#)). Together, 34 (34/98, 34.7%) patients developed brain metastasis during lifetime, including 29 with YVMA insertion and 5 with non-YVMA variants. YVMA insertion associated with a significantly higher incidence of lifetime brain metastasis (85.3% vs. 14.7%, $P=0.012$) than patients with non-YVMA variants ([Table 4](#)).

Since patient's death was a competitive factor for the development of brain metastasis, we then performed Gray's test (40) to estimate and compare the incidence of lifetime brain metastasis between the two groups. When adjusted for death, YVMA insertion still showed a significantly higher incidence of brain metastasis compared with non-YVMA variants ($P=0.002$), with an estimated 12-month brain metastasis incidence as 40.2% in the YVMA group and 3.6% in the non-YVMA group ([Figure 4](#)). While for

Table 2 Efficacy of first line chemotherapy of total patients and patients treated with pemetrexed based chemotherapy

Responses	Total patients				Patients treated with pemetrexed based chemotherapy			
	Total n (%)	YVMA n (%)	Non-YVMA n (%)	P value	Total n (%)	YVMA n (%)	Non-YVMA n (%)	P value
N	82	55	27		64	44	20	
Objective response								
CR	0	0	0		0	0	0	
PR	31 (37.8%)	17 (30.9%)	14 (51.9%)		26 (40.6%)	14 (31.8%)	12 (60.0%)	
SD	38 (46.3%)	26 (47.3%)	12 (44.4%)		26 (40.6%)	19 (43.2%)	7 (35.0%)	
PD	13 (15.9%)	12 (21.8%)	1 (3.7%)		12 (18.8%)	11 (25.0%)	1 (5.0%)	
ORR	37.8%	30.9%	51.9%	0.009	40.6%	31.8%	60.0%	0.054
DCR	84.1%	78.2%	96.3%	0.051	81.3%	75.0%	95.0%	0.085
Median PFS, (95%CI)	5.77 (5.16–6.37)	5.23 (4.02–6.45)	7.73 (5.66–9.81)	0.038	5.70 (5.16–6.24)	5.23 (3.45–7.02)	6.53 (2.52–10.55)	0.022

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; PFS, progression free survival; CI, confidential interval.

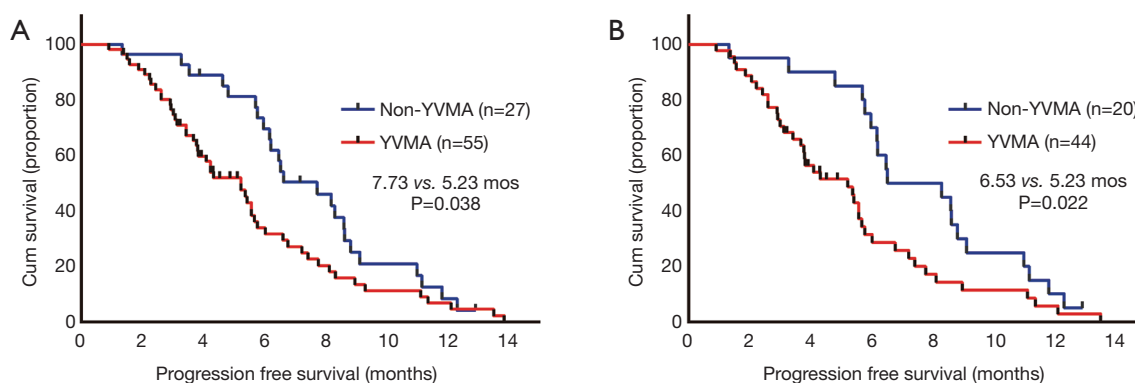


Figure 3 Comparison of PFS according to *HER2* mutation variants. (A) PFS of patients who received chemotherapy as first line treatment (n=82). (B) PFS of patients who received pemetrexed based chemotherapy as first-line treatment (n=64).

cumulative brain metastasis incidence, YVMA insertion also associated with higher incidence of 12-month brain metastasis incidence as 24.4% compared with 3.6% in the non-YVMA group with marginal significance (P=0.064).

We also compared the baseline clinicopathological features of patients with and without brain metastasis. We found that patients with baseline brain metastasis were significantly younger (median age: 50 vs. 61 years) (P=0.007), and patients with lifetime brain metastasis were also significantly younger (P=0.009). No significant differences in gender, histology, ECOG-PS, and smoking status were observed between the two groups (Table 4).

Discussion

To the best of our knowledge, this study was the first to investigate the association of *HER2* mutation variants with clinical features and efficacy of chemotherapy. We found that YVMA insertion accounted for the majority of patients with *HER2* kinase domain mutations and was associated with inferior treatment outcomes compared with patients with non-YVMA variants. We also found YVMA insertion associated with more extrathoracic disease, notably with a significantly higher incidence of baseline brain metastasis.

In our study, the incidence of *HER2* kinase domain mutations in advanced NSCLC was 4.2% for patients without common oncogenic mutations, which was in line

Table 3 Multivariate analysis for total patients and patients with pemetrexed based chemotherapy

Variables	Total patients (n=82)				Patients received pemetrexed based chemotherapy (n=64)			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Age, y								
<65	1.00	0.33			1.00	0.35		
≥65	0.757 (0.432–1.326)				0.746 (0.404–1.378)			
Gender								
Male	1.00	0.612			1.00	0.748		
Female	1.128 (0.707–1.800)				1.090 (0.645–1.840)			
Histology								
Adenocarcinoma	1.00				1.00		1.00	
Non-adenocarcinoma	1.188 (0.476–2.966)	0.712			5.219 (0.674–40.431)	0.114	3.381 (0.431–26.544)	0.247
HER2 mutation								
Non-YVMA	1.00	0.04	1.00	0.075	1.00	0.025	1.00	0.045
YVMA	1.681 (1.023–2.763)		1.578 (0.956–2.606)		1.903 (1.085–3.338)		1.789 (1.013–3.160)	
Smoking history								
Never smoker	1.00	0.328			1.00	0.779		
Ever smoker	1.286 (0.777–2.128)				1.089 (0.600–1.976)			
ECOG-PS								
0	1.00	0.77			1.00	0.872		
1–2	0.911 (0.487–1.702)				1.058 (0.532–2.107)			
Disease extent								
Intrathoracic disease	1.00	0.034	1.00	0.05	1.00	0.014	1.00	0.031
Extrathoracic disease	1.700 (1.065–2.714)	0.026	1.602 (1.0–2.568)		1.934 (1.140–3.282)		1.809 (1.055–3.101)	

ECOG-PS, Eastern Cooperative Oncology Group Performance Score; HR, hazard ratio; CI, confidential interval.

with our previous studies (11,41) and comparable to the 6% reported by Arcila *et al.* in a selected population of *EGFR/KRAS/ALK*-negative patients mainly consisting of Caucasians (42). Consistent with our previous reports (11,41), we also found that patients with HER2 kinase domain mutations were more likely to be females, younger patients, never-smokers and adenocarcinoma when

compared with patients without oncogenic gene alterations. Among them, A775_G776insYVMA accounted for 68.4% (67/98) of patients enrolled, consistent with percentage as 65–100% of *HER2*-mutated NSCLC patients reported by previous studies (9,24,41–43).

Increasing evidence has already demonstrated that driver gene mutation variants have distinct clinical features,

Table 4 Clinicopathological features of patients with and without baseline brain metastasis and lifetime brain metastasis

Characteristics	Baseline brain metastasis			Lifetime brain metastasis		
	With baseline BM, n (N%)	Without baseline BM, n (N%)	P value	With BM, n (N%)	Without BM, n (N%)	P value
Number	14	84		34	64	
Age, y						
Median	50 [36–67]	61 [33–84]	0.007	56 [36–77]	62 [3–84]	0.009
<65	12 (85.7)	59 (70.2)	0.338	30 (88.2)	41 (64.1)	0.016
≥65	2 (14.3)	25 (29.8)		4 (11.8)	23 (35.9)	
Gender			0.398			0.294
Male	6 (42.9)	47 (56.0)		21 (61.8)	32 (50.0)	
Female	8 (57.1)	37 (44.0)		13 (38.2)	32 (50.0)	
Histology			1.000			0.853
Adenocarcinoma	14 (100.0)	77 (91.7)		33 (97.1)	58 (90.6)	
Squamous carcinoma	0	1 (1.2)		0	1 (1.6)	
NOS	0	5 (6.0)		1 (2.9)	4 (6.3)	
Adenosquamous carcinoma	0	1 (1.2)		0	1(1.6)	
ECOG-PS			1.000			0.762
0	2 (14.3)	11 (13.1)		5 (14.7)	8 (12.5)	
1–2	12 (85.7)	73 (86.9)		29 (85.3)	56 (87.5)	
HER2 mutation group			0.004			0.012
YVMA	14 (100.0)	53 (63.1)		29 (85.3)	38 (59.4)	
Non-YVMA	0	31 (36.9)		5 (14.7)	26 (40.6)	
Smoking history						
Ever smoker	3 (21.4)	25 (29.8)	0.751	9 (26.5)	19 (29.7)	0.817
Never smoker	11 (78.6)	59 (70.2)		25 (73.5)	45 (70.3)	
Stage of disease			0.358			0.579
IIIB/IIIC	0	12 (14.3)		3 (8.8)	9 (14.1)	
IVA/IVB	13 (92.9)	65 (77.4)		27 (79.4)	51 (79.7)	
Recurrent	1 (7.1)	7 (8.3)		4 (11.8)	4 (6.3)	
Disease involvement			<0.001			0.005
Intrathoracic	0	49 (58.3)		10 (29.4)	39 (60.9)	
Extrathoracic	14 (100.0)	35 (41.7)		24 (70.6)	25 (39.1)	

NOS, not other specified; ECOG-PS, Eastern Cooperative Oncology Group Performance Score.

treatment outcomes, and resistance mechanisms to treatments (28,29,31–34). Recently, Hastings *et al.* further found that patients with *EGFR* L858R benefited more from immunotherapy than patients with exon 19 deletion, which could derive from the higher tumor mutation burden observed in patients with *EGFR* L858R (35). Christopoulos *et al.* also showed that the incidence of p53 co-mutation was

higher in the *EML-ALK* variant 3, which might account for its miserable prognosis and diminished outcomes to treatments (44). As for *HER2* mutations, discrepant efficacy has also been observed regarding different variants. *In vitro* studies observed that YVMA insertion has a different sensitivity to targeted therapy compared with other *HER2* mutation variants (12,45). A phase II trial investigated the

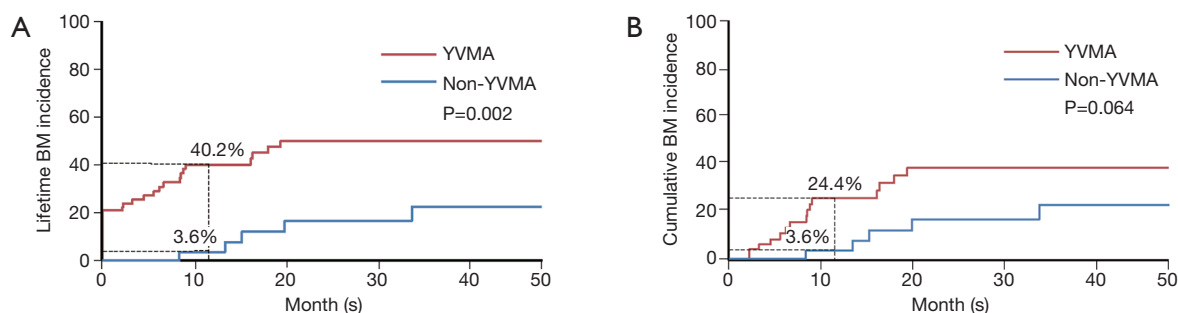


Figure 4 Comparison of lifetime brain metastases incidence and cumulative brain metastasis incidence according to *HER2* mutation variants in a total of 98 patients. The estimated 12-month brain metastases incidence was also indicated. BM, brain metastasis.

efficacy of afatinib on *HER2*-mutated NSCLC and found YVMA to be associated with superior efficacy, with 7/11 patients achieving disease control at 12 weeks compared with 0/1 patient with G776>VC (46). While in several other studies, YVMA insertion were found to associate with inferior treatment efficacy. Recently, a study by Zhang and colleagues demonstrated that G778_P780dup and G776delinsVC derived the greatest benefits from afatinib among *HER2* variants (36). Same was also observed in patients treated with pyrotinib, showing that patients with 9-bp exon 20 insertion associated with higher ORR than patients with 12-bp exon 20 insertion (19).

Currently, although targeted therapies hold great potential, chemotherapy remains the cornerstone for patients with advanced *HER2*-mutated NSCLC. The association of *HER2* mutation variants with chemotherapy and clinicopathological features needs to be further clarified. In the current study, we, for the first time, reported that patients harboring YVMA insertion displayed inferior outcomes to first-line chemotherapy, with worse ORR, DCR, and median PFS compared with non-YVMA variants. Multivariate analysis further confirmed the association of YVMA insertion with worse PFS, especially for patients received pemetrexed-based chemotherapy (HR =1.789, 95% CI, 1.013–3.160). In addition, we found extrathoracic metastases to be associated with worse PFS both in the univariate and multivariate analysis. Since patients with YVMA insertion showed a significantly higher incidence of extrathoracic metastases, the reason that patients harboring YVMA insertion benefit less from first line chemotherapy might due to their discrepancy of metastases, which further emphasized the unmet need of new therapeutic strategies for patients with YVMA insertion.

Brain metastasis is a major obstacle hindering the achievement of durable response. In our study, the incidence

of baseline brain metastasis was 14.3%, and 34.7% (34/98) patients developed brain metastasis during their lifetime. We found that YVMA insertion associated with a significantly higher incidence of baseline brain metastasis, and Gray's test further established that YVMA insertion associated with a significantly higher incidence of lifetime brain metastasis compared with non-YVMA variants. Nowadays, several second- or third-generation targeted drugs with higher blood-brain barrier (BBB) penetration capacity have shown superior efficacy in treating and postponing the onset of brain metastasis and have become the standard treatment of patients harboring EGFR/ALK alterations (47). In the current study, we, for the first time, identified the 12-month brain metastasis incidence as 40.2% in the YVMA group and 3.6% in the non-YVMA group, indicating that almost 2/5 patients with YVMA insertion might develop brain metastasis during their lifetime. In the current study, we also found that patients with baseline brain metastasis showed a shorter median PFS compared with patients without brain metastasis (median PFS: 3.1 vs. 5.9 months, $P=0.280$), even in patients with YVMA mutations alone (median PFS: 3.1 vs. 5.4 months, $P=0.632$). It is, therefore, imperative to develop targeted drugs with high BBB penetration capacity for patients of advanced NSCLC with *HER2* mutations, especially for those harboring YVMA insertion.

Several limitations must be mentioned. First, as a retrospective study, the selection bias was inevitable, and the relatively small sample size potentially limited our ability to draw affirmative conclusions. Second, although we have thoroughly reviewed medical records and collected all the information available, the real-world feature of this study might underestimate the incidence of brain metastases. Studies with a large patient number are warranted to further verify our findings. Third, *HER2* mutations were detected using ARMS-PCR, which was designed to detect

only mutations within exon19 and exon20 of HER2 kinase domain; thus rare mutations such as S310F of extracellular domain (48), V659/G660 mutation of transmembrane domain (49), and other rare *HER2* mutations which also have been demonstrated oncogenic and druggable might be missed. In addition, we did not routinely detect p53 mutation in our institute, and further studies using next-generation sequencing (NGS) are needed to further clarify the spectrum of *HER2* mutations and the incidence of co-mutations in different mutation variants.

In conclusion, our study demonstrated that the *HER2* exon 20 YVMA insertion significantly associates with aggressive disease, notably with a significantly higher incidence of both baseline and lifetime brain metastasis, together with inferior outcomes of first-line chemotherapy. Our finding indicates that more potent anti-cancer therapies with high BBB penetration capacity are needed, especially for patients with *HER2* exon 20 YVMA insertion.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the Ethics Committee of Shanghai Pulmonary Hospital, Tongji University School of Medicine. Because of the retrospective nature of the research, the requirement for informed consent was waived.

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