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ORIGINAL ARTICLE

Response rate of patients with baseline brain metastases from recently diagnosed non-small cell lung cancer receiving radiotherapy according to *EGFR*, *ALK* and *KRAS* mutation status

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Keywords
EGFR; KRAS; radiosensitivity; radiotherapy; response rate.

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Abstract

Background: Previous studies have identified that patients with *EGFR* mutations tend to have better responses to targeted therapy, as well as chemotherapy; however, the effect of genetic alterations in terms of radiotherapy (RT)-related outcomes has not been fully assessed. We studied the impact of common non-small cell lung cancer (NSCLC) genetic alterations (*EGFR*, *ALK* and *KRAS*) in relation to objective response rate (ORR) to RT in patients with brain metastases.

Methods: From 2009–2015, 153 patients with an available genotyping status were treated with whole-brain irradiation (WBI) before receiving systemic therapy. Primary outcome was ORR; secondary outcomes included intracranial progression-free survival (IPFS) and overall survival (OS).

Results: Overall, ORR was 47.1%. ORR to RT varied significantly according to molecular status: *EGFR* (64.5%) *ALK* (54.5%) *KRAS* (20%) and WT (35.4%) ($P = 0.001$). *EGFR* mutation was the only independently associated factor for response to WBI (RR 3.52 [95% CI 1.6–7.7]; $P = 0.002$). Median IPFS was 10.8 months [95% CI 8.2–13.5] overall; however, IPFS also varied significantly according to molecular status: *EGFR* (18.2 months), *ALK* (18.4 months), *KRAS* (6.0 months) and WT (8.7 months) ($P < 0.0001$). OS for *EGFR*, *ALK*, *KRAS* and WT patients was 36.6, 32.2, 15.5 and 22.4 months, respectively ($P = 0.014$). Intracranial-ORR (HR 0.4 [95% CI 0.2–0.6], $P < 0.001$) and mutation status (HR 0.7 [95% CI 0.6–0.9], $P < 0.042$) were independently associated with a higher OS.

Conclusions: RT response varies as per tumor molecular status. The presence of *EGFR* mutations favors the organ-specific response to RT, and is associated with longer OS in patients with NSCLC and BM.

Key points

- This study addressed for the first time the difference in radiotherapy-related outcomes in patients with different genotypes of non-small cell lung cancer (NSCLC) before they received systemic therapy.

- Results show that response to radiotherapy varies as per tumor molecular status, particularly *EGFR*-mutated tumors, have a favorable response to radiotherapy, contrary to *KRAS*-mutated tumors.

Introduction

Lung cancer is the main cause of cancer-related mortality worldwide, accounting for 2.07 million deaths every year, with a five-year survival which ranges between 5%–18%.[1,2] An important factor which impoverishes patient prognosis is the presence of brain metastases (BM), a relatively frequent metastatic site for lung tumors. Baseline BM are present in 10% of non-small cell lung cancer (NSCLC) patients, and this number is expected to increase with the standardization of brain imaging in asymptomatic patients, particularly in those with specific molecular alterations, such as *EGFR* mutations (*EGFR*m) or *ALK* rearrangements (*ALK*r), but also in those with elevated CEA.[3–5] Additionally, the risk of presenting BM increases throughout the course of the disease, and overall 40% of NSCLC patients will eventually present BM, although this proportion increases to 80% in particular subgroups.[4] BM usually leads to treatment failure and impaired quality of life despite treatment[4]; moreover, the prognosis of BM is poor, with a median survival of two months when treated with systemic corticosteroids alone. Whole brain irradiation (WBI) and stereotactic radiosurgery (SRS) have been the mainstay treatment for BM in NSCLC patients irrespective of histology with objective response rates (ORR) of 60%–70% for intracranial lesions and a median survival of eight months.[6–8] Nonetheless, evidence suggests that certain tumor molecular features, such as EGFR mutations, might impact the response to WBI.[9]

In spite of evidence suggesting that different-genotype tumors vary in terms of response to systemic therapy (frontline chemotherapy or tyrosine-kinase Inhibitors [TKIs]), genotype status is currently not taken into consideration in order to determine radiotherapy (RT) treatment approaches in NSCLC patients who present with BM.[10] However, it is well known that certain subsets of lung cancer patients with BM have greater objective response rates (ORR) and prolonged survival when treated with RT. One possible explanation for this variability is the previously mentioned molecular heterogeneity of NSCLC. Recent reports have suggested that *EGFR* mutation status in NSCLC patients with BM is associated with higher ORR and longer intracranial progression free survival (IPFS) compared to those with wild-type (WT) *EGFR*.[11,12] On the contrary, another report discovered that *EGFR* mutation was associated with longer OS in NSCLC patients with BM, but without a significant difference in clinical response.[13] However, both these retrospective analyses included patients who had received previous systemic therapy, and therefore the impact of the molecular feature on the radiological outcome might be confounded. The impact of other genetic alterations - such as *ALKr* and *KRAS* mutations (*KRAS*m) - in the treatment with RT as well as survival in NSCLC patients with BM has been mostly unexplored; however, a recent report in early-stage lung adenocarcinoma found that *KRAS* mutations were associated with worse local control in patients treated with stereotactic body radiotherapy (SBRT).[14]

In this study, we sought to evaluate the impact of *EGFR*m, *ALK*r and *KRAS*m in terms of ORR, IPFS and OS in patients with advanced-stage NSCLC who presented with baseline BM and were treated with WBI previous to receiving systemic therapy.

Methods

We conducted a prospective study among patients treated at the National Cancer Institute from January 2009 to June 2015. Patients with histologically-confirmed NSCLC and documented BM defined as the presence of one or more intra-axial enhancing lesions on contrast-enhanced computed tomography (CT) of the head or gadolinium-enhanced brain magnetic resonance imaging (MRI) were included (Fig. 1). Patients with a known mutational status who met the inclusion criteria were selected as our study population. Exclusion criteria included those who did not receive WBI or who did not complete the WBI therapeutic scheme (30 Gy in 10 sessions with conformal therapy technique), those without CT/MRI to assess WBI response, patients with incomplete clinical data, patients with en bloc surgical resections and those who received concomitant systemic therapy along with WBI (Fig. 1).

Variables collected for analysis included age, gender, smoking status, wood-smoke exposure (WSE), Karnofsky performance status (KPS), Eastern Cooperative Oncology Group (ECOG) performance status, disease stage (TNM, according to the American Joint Committee on Cancer Staging Manual, seventh edition[15]) mutation status (*EGFR*, *KRAS*, *ALK*, or WT), treatment for the primary tumor, number of BM, extracranial metastases (present vs. not present), and synchronous versus metachronous brain metastases, which were defined as BM diagnosed ≥2 months after the primary lung tumor. Histological type

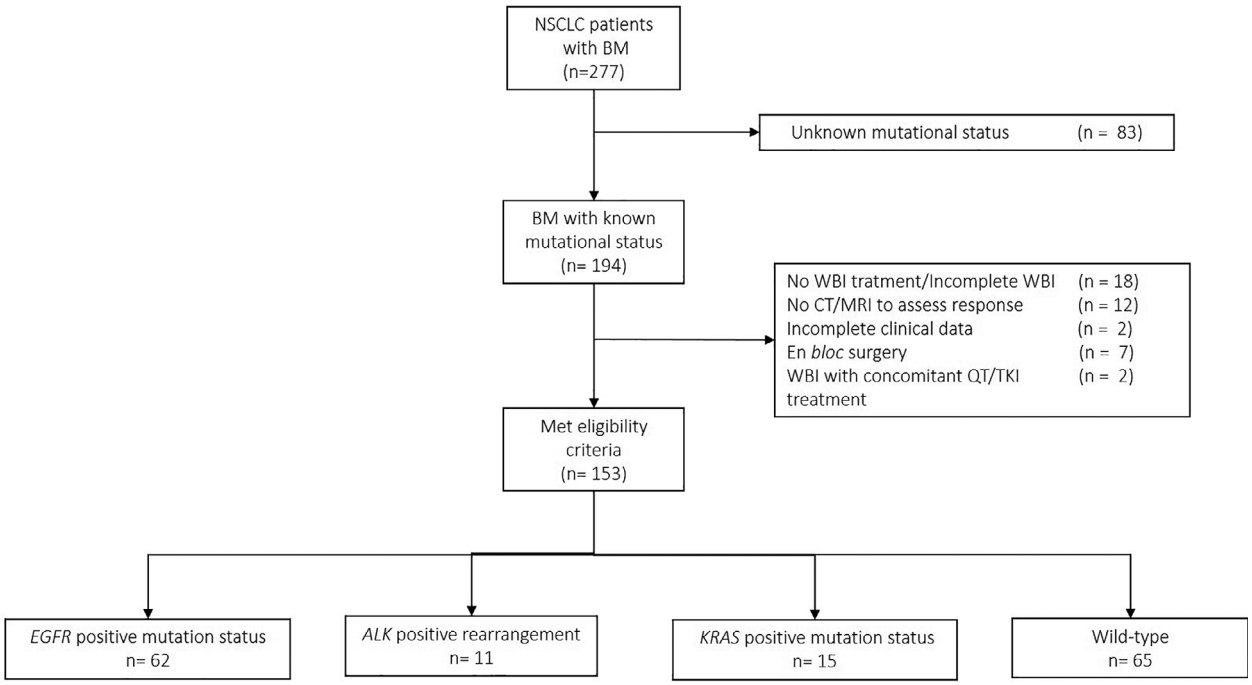

Figure 1 CONSORT diagram.

was defined conforming to IASLC/ATS/ERS International Multidisciplinary Classification of Lung Adenocarcinoma,[15] which was subdivided into lepidic predominant (LEP), acinar predominant (ACI), papillary predominant (PAP), micropapillary predominant (MIP) and solid predominant (SOL) adenocarcinoma. Tumors were grouped by architectural grading as low (LEP), intermediate (PAP or ACI), or high (MIP, SOL).[16] We calculated the recursive partitioning analysis (RPA) class as previously described.[6] Graded prognostic assessment (GPA) scores 0–4 were also calculated for all patients based on age, 50 years (1 point), 50–59 years (0.5 points), or > 60 years (0 points); KPS stratified by < 70 (0 points), 70–80 (0.5 points), or 90–100 (1 point); number of BM stratified by 1 (1 point), 2–3 (0.5 points), or > 3 (0 points); and presence or absence of extracranial metastases.[17]

Response assessment

The radiographic response of intracranial tumors was assessed by an independent blinded radiologist according to the Response Evaluation Criteria in Solid Tumors (RECIST) guideline version 1.1 by comparing the pre- and post-treatment intracranial images.[18] Any in-field tumor progression or the appearance of new malignant lesions denoted progressive disease. Objective response rate (ORR) was defined as the sum of complete and partial response.

Determination of EGFR and KRAS mutational status

Biopsies were analyzed by the pathology department for their histologic diagnosis and neoplastic cellularity quantification (>50%); they were later embedded in paraffin until processed for DNA extraction. Genomic DNA was extracted from the areas of paraffin slides using a standard procedure and a QIAamp DNA FFPE tissue kit (™QIAGEN), following the manufacturer's instructions. *EGFR* gene mutations were detected using the therascreen RGQ PCR kit (™QIAGEN, Scorpions ARMS method), which combines both the ARMS and Scorpions technologies for detecting the mutations by real-time polymerase chain reactions (PCR). Real-time PCR was performed using a Rotor-Gene Q 5plex HRM (™QIAGEN), following manufacturer's instructions.

Determination of ALK rearrangement

ALK rearrangements were identified by fluorescence in situ hybridization (Vysis LSI ALK [2p23] Dual Color, Break Apart Rearrangement Probe, Abbott Molecular). Criteria for a break-apart FISH assay to be considered positive for *ALKr* using these probes has been extensively described in previous work.[19,20]

Statistical analysis

Continuous variables were summarized as arithmetic means or medians, with standard deviation or interquartile range for descriptive purposes, and categorical variables were summarized as frequencies and percentages. Inferential comparisons were made using the one-way ANOVA or the Mann-Whitney U test, conforming to the data distribution determined by the Kolmogorov-Smirnov test. The $\chi 2$ test or Fisher's exact test were used for assessing the statistical significance of categorical variables. The ORR with 95%CI was calculated for each subgroup. We also calculated the unadjusted odds for each population subgroup and then applied a logistic regression model to predict the odds for ORR to WBRT along with their 95% CI adjusting for statistically significant covariates. The intracranial radiological progression-free survival (IPFS) was counted from the first day of brain RT to the date of radiological progression or the last radiological documentation of the intracranial disease status. The overall survival (OS) was measured from the first day of brain RT to the date of death, or last follow-up. OS and IPFS were analyzed using the Kaplan-Meier method, whereas comparisons among the subgroups were analyzed using the log rank test. For survival curve analysis, all the variables were dichotomized according to their median. Statistically significant and borderline significant variables ($P < 0.1$) were included for the adjustment in the multivariate Cox regression model and hazard ratios (HR) were calculated along with their corresponding 95% CIs as a measure of association. Statistical significance was determined as $P \leq 0.05$ using a two-tailed test. Stata software version 14 was used for all statistical analyses.

Results

Clinical characteristics

A total of 277 patients diagnosed between January 2009 and June 2015 were screened for inclusion, among these

Table 1 Treatment characteristics

	Molecular status										
	All patients		EGFR		ALK		KRAS		WT		
	n = 153		*n* = 62		*n* = 11		*n* = 15		*n* = 65		
	%	n	%	n	%	n	%	n	%	n	*P*-value
Objective response rate											
Yes	47.1%	72	64.5%	40	54.5%	6	20.0%	3	35.4%	23	**0.001**
Disease control rate											
Yes	80.4%	123	88.7%	55	90.9%	10	53.3%	8	76.9%	50	**0.012**
Intracranial therapy											
SRS	5.9%	9	3.2%	2	0.0%	0	20.0%	3	6.2%	4	
WBRT	84.3%	129	90.3%	56	81.8%	9	66.7%	10	83.1%	54	
NSG + WBRT	6.5%	10	1.6%	1	18.2%	2	6.7%	1	9.2%	6	
SRS + WBRT	3.3%	5	4.8%	3	0.0%	0	6.7%	1	1.5%	1	0.103
Median time from diagnosis of BM to cranial radiotherapy (months)											
Median (IQR)	0.49 (0.16–0.99)		0.28 (0.13–0.72)		0.72 (0.66–1.18)		1.0 (0.59. 3.91)		0.46 (0.13–1.0)		**0.001**†
Total dose (Gy)											
Median (min-max)	30 (15–46)		30 (16–46)		30 (15–30)		30 (15–37.5)		30 (16–46)		0.436†
Use of corticosteroids											
No	11.8%	18	12.9%	8	0.0%	0	13.3%	2	12.3%	8	
Yes	88.2%	135	87.1%	54	100.0%	11	86.7%	13	87.7%	57	0.660
Brain re-irradiation											
Absent	77.8%	119	80.6%	50	90.9%	10	86.7%	13	70.8%	46	
Present	22.2%	34	19.4%	12	9.1%	1	13.3%	2	29.2%	19	0.270
Total dose of brain re-irradiation (Gy)											
Median (min–max)	21.6 (16–30)		21.6 (16–30)		22.5 (22.5–22.5)		25.8 (21.6–30)		21.6 (16–30)		0.877†

BM, brain metastases; KPS, Karnofsky performance status; ECOG PS, European Clinical Oncological Group performance status; EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog; ALK, anaplastic lymphoma kinase gene translocations; TKI, tyrosine kinase inhibitor; SRS, stereotactic radiosurgery; WBRT, whole brain radiation therapy; NSG, neurosurgical resection; S.D., standard deviation. †Kruskal-Wallis test *P*-value.

194 had available information in terms of the mutational status of *EGFR*, *ALK* and *KRAS*. A total of 41 patients were excluded as described in Fig. 1. Thereafter, 153 patients met the eligibility criteria and were included in the final analysis (Fig. 1).

The following molecular alterations were identified: *EGFRm* in 40.5% of samples, *KRASm* in 9.8% of samples, and *ALKr* in 7.2% of samples; WT status for these aberrations was identified in 42.5% of our study population. Median follow-up was 17.7 months (range: 1.18–74.1 months). Mean age was 56.1 ± 12.2 years, 61.4% were female, 38.6% were men and 95.4% had adenocarcinoma histology (Table S1). Higher rates of tobacco exposure were found in the *KRASm* (86.7%) and WT (56.9%) subgroups compared to the *EGFRm* patients (24.2%) or *ALKr* patients (36.4%) ($P < 0.001$). By contrast, *EGFRm* and *ALKr* patients were more likely to report wood-smoke exposure (WSE) compared to *KRASm* and WT patients (61.3%, 45.5% vs. 6.7% and 24.6% respectively; $P < 0.001$). There was a significant association between the extent of extracranial disease and positive genetic alteration status compared with WT patients ($P = 0.002$).

Treatment response analysis

The overall objective response rate (ORR) was 47.1% (7.3% complete response and 39.8% partial response). The overall disease control rate (DCR) was 80.4%, progressive disease was seen in 19.6% of the patients. The ORR was significantly higher among patients with *EGFRm* (64.5%) and *ALKr* (54.5%) compared with *KRASm* (20.0%) and WT patients (35.4%) ($P = 0.001$). Likewise, DCR were higher among patients harboring *EGFRm* (88.7%) and *ALKr* (90.9%) compared with *KRASm* (53.3%) and WT patients (76.9%) ($P = 0.012$) (Table 1). *EGFRm* status was associated with higher decreases in tumor size after WBI, and this decrease was associated with an increase in progression-free survival (Fig. 2(a),(b)) and OS (Fig. 2(c),(d)).

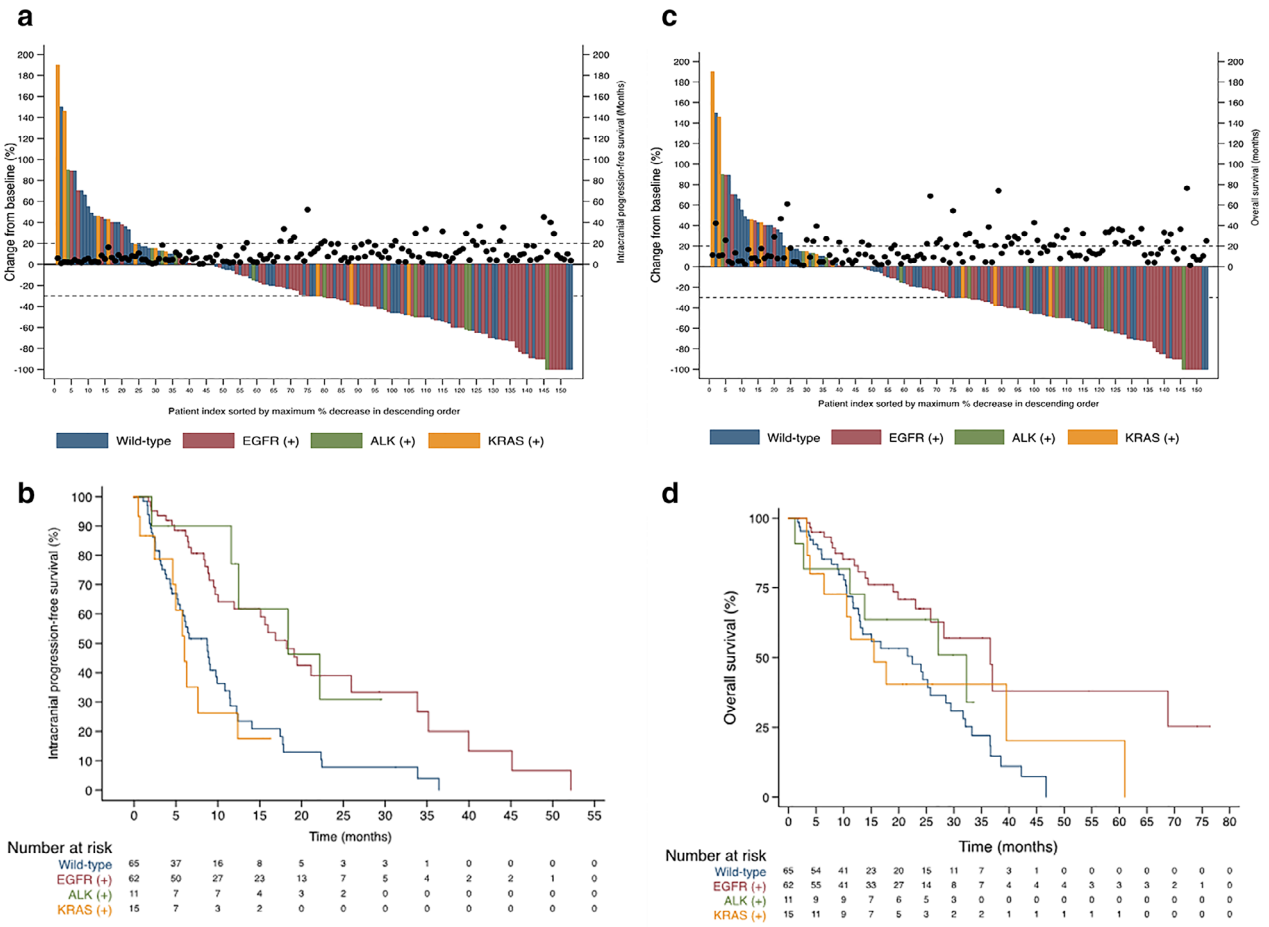

Figure 2 (**a**) Waterfall plot of the percentage of change from baseline in tumor size (bars) and the IPFS (dots) among patients by mutation status. (**b**) Kaplan-Meier curve for IPFS according to mutation status. (**c**) Waterfall plot of the percentage of change from baseline in tumor size (bars) and the OS (dots) among patients by mutation status. (**d**) Kaplan-Meier curves for OS according to mutation status.

The clinical characteristics associated with ORR are summarized in Table 2. *EGFRm* was the only factor significantly associated with ORR among our population. In the univariate analysis, *EGFRm* was significantly associated with ORR to WBI (RR 3.32 [95% CI 1.60–6.87], $P = 0.001$). On the multivariate analysis, *EGFRm* status was the only independent predictive factor associated with ORR (RR 3.52, [95% CI 1.61–7.72], $P = 0.002$). Thus, a patient harboring an *EGFRm* was almost four times more likely to respond to WBI than other patients analyzed in this study.

Intracranial progression-free survival

Median IPFS after WBI was 10.8 months (95%CI 8.2–13.5). Factors which positively influenced IPFS in the univariate analysis included never-smoker status

Table 2 Univariate and multivariate analysis of factors associated with intracranial objective response of BM treatment in 153 evaluable patients

		Univariate analysis			Multivariate analysis		
	Overall response rate (95% CI)	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Overall	47.1% (39.1%– 55.1%)						
Gender							
Female	46.8% (36.6%– 57.0%)	0.97	(0.5–1.9)	0.938			
Male (reference value)	47.5% (34.5%– 60.4%)	1.00	-	-			
Median age							
≤60 (reference value)	47.8% (37.3%– 58.2%)	1.00	-	-			
60+	46.0% (33.5%– 58.5%)	0.93	(0.49–1.78)	0.832			
Smoking exposure							
Never	50.0% (39.2%– 60.8%)	1.3	(0.68–2.47)	0.423			
Ever	43.5% (31.6%– 55.4%)	1.00	-	-			
Wood-smoke exposure							
Absent	43.0% (32.8%– 53.2%)	0.66	(0.34–1.27)	0.214	1.17	(0.55–2.48)	0.675
Present (reference value)	53.3% (40.5%– 66.2%)	1.00	-	-			
ECOG PS							
<2	46.6% (37.5%– 55.7%)	0.92	(0.43–1.97)	0.839			
2+ (reference value)	48.6% (31.6%– 65.5%)	1.00	-	-			
KPS at BM diagnosis							
<70	37.5% (1.3%– 73.7%)	0.65	(0.14–2.84)	0.570			
≥70 (reference value)	47.9% (39.7%– 56.2%)	1.00	-	-			
Extracranial metastases							
Absent	44.4% (32.0%– 56.9%)	0.83	(0.43–1.59)	0.589			
Present (reference value)	48.9% (38.4%– 59.4%)	1.00	-	-			
Number of BM							
<3	45.2% (33.6%– 56.8%)	0.86	(0.45–1.64)	0.662			
3+ (reference value)	48.8% (37.6%– 59.9%)	1.00	-	-			
Mutation status							
WT (reference value)	35.4% (23.6%– 47.2%)	1.00	-	-	1.00	-	-
EGFR positive	64.5% (52.4%– 76.6%)	3.32	(1.60–6.87)	**0.001**	3.52	(1.61–7.72)	**0.002**
ALK rearrangement	54.5% (23.4%– 85.7%)	2.19	(0.60–7.96)	0.234	2.26	(0.61–8.34)	0.218
KRAS positive	20.0% (0.0%– 41.1%)	0.45	(0.11–1.78)	0.260	0.44	(0.11–1.74)	0.245
RPA class							
I	50.0% (30.25–69.8%)	1.66	(0.32–8.46)	0.538			
II	47.1% (38.0%– 56.1%)	1.48	(0.33–6.48)	0.602			
III (reference value)	37.5% (1.3%– 73.7%)	1.00	-	-			
GPA class							
0–1 (reference value)	44.1% (27.0%– 61.2%)	1.00	-	-			
1.5–2.0	46.5% (34.7%– 58.3%)	1.1	(0.48–2.50)	0.820			
2.5–3.0	51.2% (35.9%– 66.4%)	1.32	(0.53–3.27)	0.539			
3.5–4.0	40.0% (8.4%–88.4%)	0.84	(0.12–5.71)	0.862			

BM, brain metastases; KPS, Karnofsky performance status; ECOG PS, European Clinical Oncological Group performance status; EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog; ALK, anaplastic lymphoma kinase gene translocations; WT wild-type; RPA, recursive partitioning analysis; GPA, graded prognostic assessment; ADC, adenocarcinoma.

Table 3 Univariate and multivariate analysis of factors associated with intracranial progression-free survival in 153 evaluable patients

	Univariate analysis			
	Median (months)	*P*-value	95% CI	*P*-value
Overall	10.8 (8.2–13.5)			
Gender				
Female	10.0 (6.6–13.4)			
Male	11.5 (8.5–14.4)	0.742		
Median age (years)				
<60	11.5 (5.9–16.9)			
≥60	9.9 (5.3–14.5)	0.475		
ECOG PS at diagnosis				
0–1	10.0 (6.9–13.1)			
≥2	10.8 (8.0–13.6)	0.405		
Histology				
Adenocarcinoma	11.4 (8.9–13.9)			
Other	10.8 (NR)	0.173	0.5 (0.2–1.5)	0.231
Architectural grade				
Low and intermediate	12.4 (9.7–15.0)			
High	9.0 (7.4–10.6)	0.994		
Smoking exposure				
Never-smoker	15.1 (8.3–21.9)			
Ever-smoker	8.9 (5.6–12.1)	**0.011***		
Wood-smoke exposure				
Present	16.0 (9.2–22.8)			
Absent	8.9 (7.7–10.2)	**0.046**	0.2 (0.5–1.1)	0.164
KPS at BM diagnosis				
<70	2.8 (0.5–5.1)			
≥70	11.6 (9.4–13.8)	**<0.001**	0.3 (0.1–0.8)	**0.014**
RPA group				
I	15.1 (6.9–23.2)			
II	10.8 (8.4–13.2)			
III	2.8 (0.5–5.1)	**<0.001**	1.5 (0.8–3.0)	0.209
GPA group				
0–1	8.8 (5.1–12.6)			
1.5–2.0	10.0 (7.0–13.0)			
2.5–3.0	15.1 (5.2–25.1)			
3.5–4.0	NR (NR)	0.632		
Extracranial metastases				
Absent	9.5 (7.1–11.9)			
Present	14.1 (7.9–20.3)	0.672		
Number of BM				
1	8.9 (8.6–9.3)			
2 to 3	16.0 (9.5–22.5)			
>3	10.8 (8.4–13.3)	0.114	0.4 (0.7–1.2)	0.414
Mutational status				
WT (reference value)	8.7 (5.8–11.7)			
EGFR positive	18.2 (14.0–22.4)			
ALK positive	18.4 (6.7–30.1)			
KRAS positive	6.0 (4.4–7.7)	**<0.001**	0.9 (0.7–1.1)	0.315
Carcinoembryonic antigen at diagnosis (ng/mL)				
<20	15.1 (8.5–21.8)			
≥20	9.5 (8.4–10.6)	**0.030**	1.5 (0.9–2.4)	0.075

ALK, anaplastic lymphoma kinase gene translocations; BM, brain metastases; ECOG PS, European Clinical Oncological Group performance status; EGFR, epidermal growth factor receptor; KPS, Karnofsky performance status; KRAS, Kirsten rat sarcoma viral oncogene homolog; NSG, neurosurgical resection; SRS, stereotactic radiosurgery; WBRT, whole brain radiation therapy. *Breslow test *P*-value.

Table 4 Univariate and multivariate analysis of factors associated with overall survival in 153 evaluable patients

	Univariate analysis			
	Median (months)	*P*-value	HR (95% CI)	*P*-value
OVERALL	25.8 (21.2–30.4)			
Gender				
Female	27.2 (23.7–30.6)			
Male	24.3 (16.9–31.7)	0.819		
Median age (years)				
<60	27.1 (20.3–33.9)			
≥60	25.7 (12.7–38.7)	0.492		
ECOG PS at diagnosis				
0–1	25.7 (21.4–30.1)			
≥2	25.8 (8.9–42.7)	0.630		
Histology				
Adenocarcinoma	25.8 (21.6–29.9)			
Other	15.0 (0.0–36.3)	0.735		
Architectural grade				
Low and intermediate	28.6 (19.6–37.5)			
High	17.7 (5.7–29.7)	0.071		
Smoking exposure				
Never-smoker	28.6 (21.9–35.2)			
Ever-smoker	24.3 (12.7–35.9)	0.556*		
Wood-smoke exposure				
Absent	24.0 (19.4–28.7)			
Present	32.3 (21.1–43.5)	0.075	0.7 (0.4–1.2)	0.221
KPS at BM diagnosis				
< 70	3.9 (0.0–17.2)			
≥70	28.6 (22.5–34.6)	**<0.001**		
RPA group				
I	36.6 (7.3–65.8)			
II	27.1 (22.2–32.1)			
III (reference value)	3.9 (0.0–17.2)	**<0.001**	2.8 (1.4–5.4)	**0.003**
GPA group				
0–1	18.9 (0.0–38.0)			
1.5–2.0	27.1 (19.5–34.8)			
2.5–3.0	29.4 (20.1–38.8)			
3.5–4.0	17.7 (8.5–25.9)	0.526		
Extracranial metastases				
Absent	24.0 (13.6–34.5)			
Present	28.2 (24.5–31.9)	0.720		
Number of BM				
1	19.8 (10.2–29.5)			
2+	36.9 (29.4–44.5)			
>3	25.7 (22.1–29.4)	0.200		
Mutational status				
WT (reference value)	22.4 (10.4–34.5)			
EGFR positive	36.6 (26.1–47.1)			
ALK positive	32.2 (4.7–26.3)			
KRAS positive	15.5 (4.7–26.3)	**0.014**	0.7 (0.6–0.9)	**0.043**
Intracranial objective response				
Present	32.1 (27.4–36.8)			
Absent	11.8 (4.8–18.7)	**<0.001**	0.4 (0.2–0.6)	**<0.001**
Carcinoembryonic antigen at diagnosis (ng/mL)				
<20	32.3 (19.1–45.5)			
≥20	23.0 (14.2–31.9)	**0.089**	0.4 (0.8–2.1)	0.377

ALK, anaplastic lymphoma kinase gene translocations; BM, brain metastases; ECOG PS, European Clinical Oncological Group performance status; EGFR, epidermal growth factor receptor; KPS, Karnofsky performance status; KRAS, Kirsten rat sarcoma viral oncogene homolog; NSG, neurosurgical resection; SRS, stereotactic radiosurgery; WBRT, whole brain radiation therapy. *Breslow test *P*-value.

(15.1 months vs. 8.9 months; $P = 0.011$), wood-smoke exposure (16.0 months vs. 8.9 months; $P = 0.046$), good CNS Karnofsky performance status (11.6 months vs. 2.8 months; $P < 0.001$), lower RPA class (I vs. II vs. III) (15.1 months vs. 10.8 months vs. 2.8 months; $P < 0.001$) and presence of an *EGFRm* or *ALKr* vs. *KRASm* or WT status (18.2 months vs. 18.4 months vs. 6.0 months vs. 8.7 months; $P < 0.001$) (Fig. 2(c),(d)). The multivariate analysis showed that a better Karnofsky performance status (<70) was associated with IPFS (HR: 0.3, 95%CI: 0.1–0.8; $P = 0.014$) (Table 3).

Overall survival

The median OS was 25.8 months (95% CI 21.2–30.4). However, median OS varied significantly as per molecular status (*EGFRm*: 36.6 months, *KRASm*: 15.5 months, *ALKr*: 32.2 months and WT: 22.4) ($P = 0.014$) (Fig. 2(d)). Several factors positively influenced OS in the univariate analysis, including Karnosfky performance status at BM diagnosis ≥70 (28.6 months vs. 3.9 months; $P < 0.001$), lower RPA class (I vs. II and III) (36.6 months vs. 27.1 months vs. 3.9 months; $P < 0.0001$), and intracranial ORR (32.1 months vs. 11.8 months, $P = 0.001$). In the multivariate Cox proportional regression analysis, a higher RPA (>3) was associated with a higher risk of death (HR: 2.8 [95% CI 1.4–5.4]; $P = 0.003$). By contrast, WT mutation status (HR: 0.7 [95% CI 0.6–0.9]; $P = 0.042$) and an intracranial ORR (HR: 0.4 [95% CI 0.2–0.6]; $P < 0.001$) were associated with a lower risk of death (Table 4).

Discussion

Most NSCLC patients will develop BM during disease course, which considerably decrease survival and quality of life. Factors which are independently associated with a poor prognosis for patients with BM include age (<65 years), poor ECOG performance status, hiliar lymph node involvement, an increasing primary tumor size, and lymphovascular space invasion.[21] Radiotherapy is considered the cornerstone treatment in NSCLC patients who present with BM; however, response rates range widely, and we currently lack predictive tools to assess which patients will most benefit from this intervention.[12] Interestingly, genetic aberrations are well characterized within the advanced-stage NSCLC population.[14] The impact of these genetic aberrations in terms of patient outcomes has been extensively studied for systemic interventions. For example, *EGFR*-mutated patients included in the IPASS study had better response to targeted therapy with gefitinib compared to wild-type *EGFR* patients (71.2% vs. 1.1%), but also had a higher response to taxane-based chemotherapy schemes (47.3% vs. 23.5%, respectively), highlighting the differential tumor biology between these molecular subtypes and its impact on clinical outcomes.[22] However, the genetic profile is not currently taken into consideration in order to drive radiotherapy recommendations.[14]

To the best of our knowledge this is the first study to investigate and compare the impact of genetic alterations on treatment response and survival of patients with BM from NSCLC treated with WBI, previous to the administration of systemic therapy. Our results show that response to WBI was significantly higher in patients with *EGFRm* and *ALKr*, compared to those who present *KRASm* and with wild-type patients. These data are consistent with previous reports that identify a higher response to RT for *EGFRm* patients. Nonetheless, in previous studies, a large proportion of the study population received targeted therapy or chemotherapy concurrent with the radiotherapy, and therefore could have impacted these results.[6,12]

Although EGFR overexpression is generally associated with radioresistance in cancer, *EGFR* mutations in NSCLC have been shown to confer radiosensitivity in vitro.[13] NSCLC cell lines with mutant *EGFR* exhibit higher sensitivity to radiation, evidenced by increased apoptosis or delayed double-strand DNA break repair.[11,12,23] Furthermore, upon irradiation, the clonogenic survival of overexpression of either △E746–E750 deletion or L858R form of EGFR in immortalized human bronchial epithelial cells is reduced by up to 1000-fold.[24]

A retrospective analysis of 63 patients with BM from lung adenocarcinoma who were treated with WBI found an ORR of 46.0%, with higher response rates in *EGFRm* patients (54.0%) compared with WT patients (24.0%).[9] Similarly, Lee *et al.* reported higher ORR to WBI in patients with *EGFRm* compared to WT patients (80.0% vs. 46.0%; $P = 0.037$).[12] Hsiao *et al.* described a similar pattern in terms of response rates in *EGFRm* and WT patients (84.0% vs. 48.0%, $P = 0.002$).[11] Meanwhile, Stanic *et al.* showed that *EGFRm* positive patients have a much longer median time to CNS progression compared to wild-type patients (25.8 vs. 11.8 months; $P = 0.002$).[25]

In a small cohort of NSCLC patients with BM, Johung *et al.* demonstrated that tumors with positive *EGFRm* and *ALKr* translocations have superior control rates, suggesting that both subtypes are radiosensitive genotypes, compared to *KRASm* and WT tumors.[26] In agreement with previous reports, we found an ORR of 64.5%, 54.5% and 35.4% for *EGFRm*, *ALKr* and WT patients. However, to the best of our knowledge, this is the first prospective observation in terms of *KRASm* patients without systemic therapy, and our data suggests that this NSCLC genotype has a lower response rate to WBI (20.0%) compared to other common mutations and WT patients; however, this did not reach statistical significance in the multivariate analysis, probably due to a limited sample size. Our data also showed that

KRASm was an independent prognostic factor of poor response rate to WBRT in NSCLC patients. This finding is in line with previous reports. A retrospective study which included 157 NSCLC patients who received RT for brain metastases showed in the multivariate analysis that *KRAS* G12V or G12C status was associated with both poor response rate (OR: 0.1; $P < 0.0001$) and shorter OS (OR: 3.41; $P < 0.0001$).[27] It has been suggested that different *KRAS* amino acid substitutions could affect different downstream signaling pathways. A previous report showed that *KRAS* G12C was associated with reduced response to cisplatin and increased sensitivity to taxol and pemetrexed, whereas G12V was more resistant to pemetrexed. Thus, it is likely that WBI could have different effects due to the radiosensitivity of the cells, which might be molecularly mediated.[28]

In the current study, we demonstrated that *ALKr* was associated with a higher response rate to WBI in NSCLC. Of note, the PFS and OS of the *ALKr* population were lower than in previous reports.[29,30] This discrepancy might be explained because less than 40% of the *ALKr* population received targeted therapy. Nonetheless, we demonstrated that *ALKr* populations have a longer IPFS and OS. However, the effect of *ALKr* in relation with WBI response remains unclear and further analyses are required.

We showed that intracranial progression was different in accordance with molecular status favoring *ALKr* and *EGFRm* over WT and *KRASm* populations. This benefit might be in relation with targeted therapies, particularly EGFR-TKIs, as has been previously reported.[31] Although we did not find a clear benefit of the combination of EGFR-TKI and RT in OS, it has been postulated that a better penetrance of TKIs after RT is in order, probably due to the rupture of the blood-brain barrier.

Another important question which should be answered in future studies is whether *EGFRm* patients with BM should be treated with lower doses of RT, and *KRASm* as well as WT patients with BM should be allocated to higher doses WBI whenever possible; speculatively, this could prevent unnecessary toxicity in the first and improve outcomes in the latter.

Although this study presents several strengths, including its prospective design and the fact that none of the patients were receiving systemic therapy at the time of WBI, all data should be interpreted in light of its limitations, particularly, regarding *KRASm* and *ALKr* population sample size. It is important to mention that *KRASm* frequency appears to be lower compared to other regions, which is in fact a feature of NSCLC in Latin America, where a high proportion of the cases are not associated with a positive smoking history, and therefore *KRASm* frequency tends to be lower. This same phenomenon also drives the higher proportion of EGFRm patients, which in México is reported to have a much higher frequency compared to other world regions.[32] Therefore, the prevalence of *EGFR*, *KRAS* and *ALK* mutations reflects a Hispanic population.[32,33] It is interesting to note that currently the tumor molecular profile is not considered in order to make therapeutic decisions in terms of RT, and in light of the evidence provided in this study as well as previous retrospective trials this might be in need of further exploration. Further, targeted therapies, specifically EGFR-TKIs, might have a benefit in increasing therapeutic response to WBI in NSCLC patients harboring an *ALKr* or *EGFRm*. However, the benefit of EGFR-TKIs in addition to WBI in terms of OS remains unclear. Interestingly, third-generation TKIs, which feature a higher CNS penetration, might eventually drive WBRT out of the clinical practice context. However, global access and affordability must be met for this to occur, and therefore a large proportion of NSCLC patients will continue to receive RT as a therapeutic option for BM.

In conclusion, patients with NSCLC who present with brain metastases have varied responses to WBI, and these are affected by the molecular alterations which characterize the tumor. *EGFRm* is an independent prognostic factor to WBI response in NSCLC, and patients with these characteristics have a significantly longer IPFS. On the contrary, *KRASm* patients have significantly lower ORR; however, samples were limited in this patient subgroup. The effect of this aberration should be further studied in the context of RT-based treatments in order to draw more robust conclusions.

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Supporting Information

Additional Supporting Informationmay be found in the online version of this article at the publisher's website:

Table S1 Baseline characteristics for the entire study population

