Article

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Efficacy of PD-1 Inhibitors Combined with Anti-Angiogenic Therapy in Driver Gene Mutation Negative Non-Small-Cell Lung Cancer with Brain Metastases

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Objective: Anti-angiogenic therapy has proven effective in non-small-cell lung cancer (NSCLC) patients. The purpose of this study was to evaluate the efficacy of programmed cell death protein 1 (PD-1) inhibitors combined with anti-angiogenic therapy in patients with driver gene mutation negative NSCLC and brain metastases (BMs).

Methods: A retrospective analysis was performed on NSCLC BMs in patients without driver gene mutations who received PD-1 inhibitors. Two groups, receiving either PD-1 inhibitor monotherapy or PD-1 inhibitor plus anti-angiogenesis therapy, were identified. The primary endpoints were overall survival (OS) and intracranial progression-free survival (iPFS). The secondary endpoints were safety, intracranial objective response rate (iORR) and intracranial disease control rate (iDCR).

Results: 113 NSCLC patients were included, 51 (45.1%) in the PD-1 inhibitor monotherapy group and 62 (54.9%) in the PD-1 inhibitor plus anti-angiogenesis therapy group. The median follow-up time was 26.2 months. OS was higher in the combination therapy cohort than in the monotherapy cohort (OS: 21.4 *vs.* 11.8 months; $p = 0.004$), with no significant difference in iPFS ($p = 0.088$). Moreover, the PD-1 inhibitor + anti-angiogenic therapeutic regimen exhibited the preferred iDCR ($p = 0.005$) but not the iORR ($p = 0.121$). There was no significant difference in the incidence of grade 3–4 adverse events between the two groups. In multivariate Cox regression analysis, PD-1 inhibitor therapy combined with anti-angiogenic treatment ($p = 0.003$) was an independent prognostic indicator of OS.

Conclusions: Combining PD-1 inhibitor therapy with anti-angiogenic treatment significantly improves the OS of driver gene mutation negative NSCLC patients with BMs.

Keywords: non-small-cell lung cancer; brain metastases; PD-1 inhibitors; anti-angiogenic

Background

Lung cancer has high morbidity and mortality around the world [1]. Non-small-cell lung cancer (NSCLC) is the most common pathological type, accounting for around 85% of all lung cancers. The majority of NSCLC patients are already in an advanced stage when diagnosed, and the 5-year survival rate is less than 15% [2]. The brain is the most common site for metastasis, with about 40% of NSCLC patients developing brain metastases (BMs) during disease progression, and about 10% of newly-diagnosed NSCLC patients already having BMs [3]. For BMs patients, local treatment, including surgery, whole brain radiotherapy (WBRT) and stereotactic radiosurgery (SRS), remains mainstream therapy. However, these patients generally have a poor prognosis, low quality of life, and short survival [4]. With the continued development of new anti-tumor drugs and the wide application of genetic testing techniques, the treatment of advanced NSCLC has made remarkable progress in recent years. For NSCLC patients with driver gene mutations, molecular targeted therapy with specific gene mutations is currently recognized as the best therapeutic approach [5]. As for driver gene mutation negative NSCLC patients, with the advent of immunotherapy, immune checkpoint inhibitors (ICIs) have demonstrated excellent efficacy in advanced and locally advanced NSCLC patients [6–9], changing the traditional therapeutic options.

Furthermore, due to ICIs being widely used in clinical practice, existing data have confirmed that ICIs monotherapy is effective in NSCLC or melanoma patients with BMs, and reduces the incidence of BMs [8–11]. Nevertheless, the challenges of acquired resistance still exist, and the most effective therapeutic approach for BM patients remains controversial. Thus, several therapeutic regimens, such as ICIs

combined with chemotherapy [12] or with anti-angiogenic regimes [13], and programmed cell death-Ligand 1 (PD-L1) inhibitors combined with anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) [14] are under study.

Angiogenesis plays a crucial role in tumor survival and metastasis. Previous studies and real-time imaging by multiphoton laserscanning microscopy (MPLSM) in a BM mouse model indicate that angiogenesis plays an important role in the early formation of lung cancer metastases [15]. As a result, anti-angiogenic therapy is a promising regimen that primarily destroys blood vessel supply and impedes tumor growth. First, anti-angiogenic drugs change the tumor's microenvironment from one that is immunosuppressive to one that is permissive to the immune system. In other respects, ICI-activated immunity promotes anti-angiogenic effects by reducing vascular endothelial growth factor (VEGF) expression and alleviating hypoxia [16]. Next, anti-angiogenic therapies targeting VEGF boost T cell trafficking into the tumor and decrease immunosuppressive cytokines, which may assist in overcoming resistance to ICIs [17]. This combination therapy has been proven by relevant clinical studies. IMpower150 indicated that atezolizumab and bevacizumab significantly improve the progression-free survival (PFS) and overall survival (OS) of non-squamous NSCLC patients [11,18]. Though results from the IB trial of Sintilimab plus Anlotinib also showed advantages [19], no analysis of survival outcomes in the BM cohort was shown. Moreover, most clinical studies exclude both untreated and symptomatic BMs patients, causing underrepresentation of these patients in study outcomes.

Hence, it is critical to determine whether patients with real-world NSCLC BMs can benefit from adjuvant anti-angiogenic therapy. The primary objective of this retrospective study was to assess the effect of programmed cell death protein 1 (PD-1) inhibitors combined with anti-angiogenic therapy on driver gene mutation negative NSCLC patients with BMs.

Methods

Patients

From December 2017 to December 2022 in the Second Affiliated Hospital of Nanchang University and Jiangxi Provincial People's Hospital (The First Affiliated Hospital of Nanchang Medical College), according to the 8th edition of American Joint Committee on Cancer (AJCC) cancer staging system, 1239 patients were pathologically or cytologically diagnosed with NSCLC (stage IV). Altogether, 517 patients received PD-1 inhibitor therapy. Among them, 209 patients were diagnosed with BM by craniocerebral magnetic resonance imaging (MRI). Patients were enrolled according to the following inclusion and exclusion criteria. The inclusion criteria were: (1) patient was older than age 18 years; (2) patient had detailed radiographic evaluations, such as chest or abdomen computed tomography (CT) scans, emission computed tomography (ECT) scans of bone, and brain MRI, before and during treatment; (3) patient had intracranial and systemic metastases with at least one measurable lesion; (4) patient had received no previous ICIs therapy; (5) patient displayed no sensitive epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) driver gene alteration. The exclusion criteria were: (1) patient developed BM after or during immunotherapy; (2) patient received only 1–2 courses of ICIs; (3) patient was diagnosed with leptomeningeal metastasis by cerebrospinal fluid cytology (CSF); (4) patient required long-term steroid use for autoimmune diseases; (5) patient had no complete follow-up records or necessary imaging examinations. We defined active BMs as newly diagnosed, recurrent BMs after prior regional brain treatment and symptomatic BMs. Retrieval of electronic imaging data of patients from the medical record system for evaluation of efficacy. When possible, patients were followed up by telephone or face-to-face interviews.

Treatment and Evaluation

The ICIs used are anti-PD-1 antibodies, incorporating Nivolumab (CAS NO.946414-94-4, Bristol-Myers, America, 3 mg/kg every 2 weeks); Pembrolizumab (CAS NO.1374853-91-4, Merck & Co., Inc., America, 2 mg/kg every 3 weeks); or Sintilimab (CAS NO.2072873-06-2, Suzhou Innovent Biologics Co., Ltd., China), Tislelizumab (CAS NO.1858168-59-8, Hangzhou MolCore BioPharmatech Co., Ltd., Hangzhou, China) and Camrelizumab (CAS NO.1798286-48-2, Jiangsu Hengrui Pharmaceuticals Co., Ltd., China) (all 200 mg every 3 weeks). Anti-angiogenesis drugs included Anlotinib (CAS NO.1210828-44-6, Jiangsu Chia Tai-Tianqing Pharmaceutical Co., Ltd., China) once daily (8 mg, 10 mg or 12 mg) for 14 consecutive days, a three-week regime; and Bevacizumab (CAS NO.216974-75-3, Roche Pharma Ltd., Switzerland) (7.5 mg/kg every 3 weeks). During immunotherapy, patients were required to receive at least four cycles of anti-angiogenic therapy for enrollment.

The study used the National Cancer Institute's generic term Adverse Events (AEs) Criteria (Version 4.0) to assess safety and tolerability. MRI and CT scans were done every 6–8 weeks to estimate the tumor response to ICIs until progression. Tumor response was evaluated using response evaluation criteria in solid tumors (RECIST) 1.1. Each observation was divided into complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). The intracranial objective response rate (iORR) was defined as the proportion of patients with intracranial CR (iCR) and intracranial PR (iPR). The intracranial disease control rate (iDCR) referred to the ratio of iCR, iPR, and intracranial SD (iSD) cases. Intracranial PFS (iPFS) was defined as the time from the initiation of PD-1 inhibitor combination or monotherapy to the time of intracranial PD

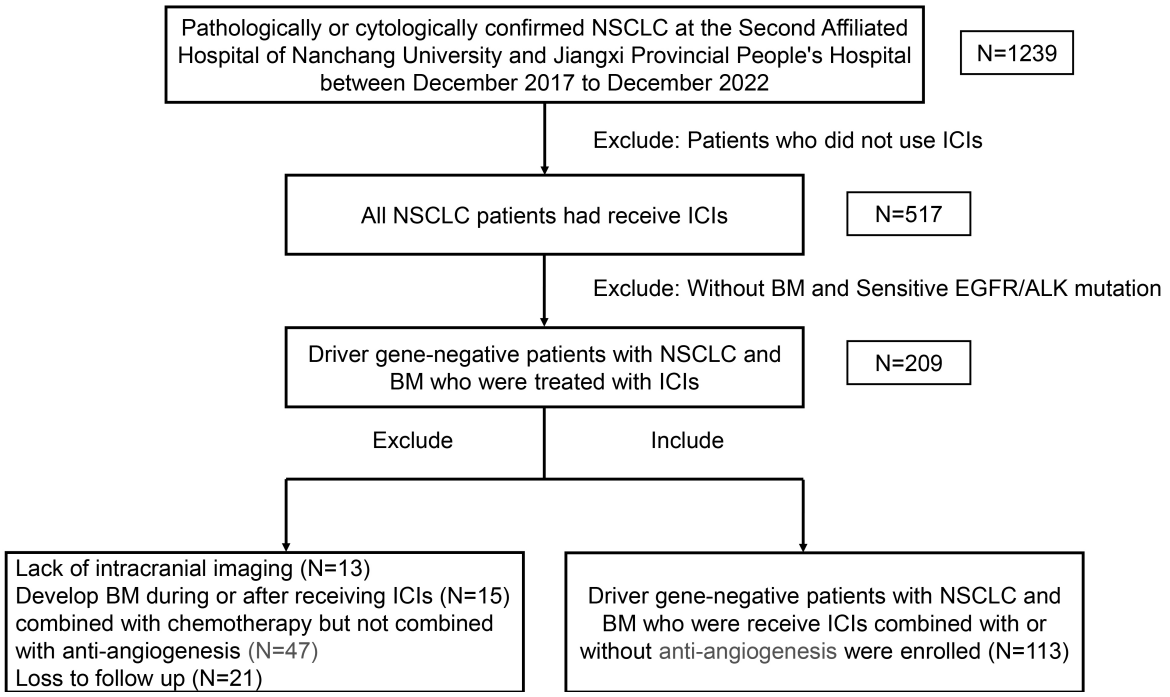

Fig. 1. Flowchart of the patient queue. NSCLC, Non-small-cell lung cancer; BM, Brain metastases; ICIs, Immune checkpoint inhibitors; EGFR, Epidermal growth factor receptor; ALK, Anaplastic lymphoma kinase.

(iPD) (confirmed by craniocerebral MRI) or death. Patients who didn't progress or died had their follow-up censored. PFS was defined as the time from the initiation of PD-1 inhibitor combination or monotherapy to the time of PD or death. OS was defined as the time from the first combination or single dose to death or the last follow-up of a surviving patient.

The expression of PD-L1 in tumor cells was obtained from the lung pathology reports. PD-L1 expression was assessed by the Tumor Score Index (TPS), where TPS $<1\%$ was defined as PD-L1 negative expression and PD-L1 positive was defined as borderline TPS $\geq 1\%$. Additionally, Next-generation sequencing (NGS) was used to detect gene mutation status.

Statistical Analysis

The baseline characteristics, including iORR and iDCR, were compared between the PD-1 inhibitor monotherapy and PD-1 inhibitor plus anti-angiogenesis therapy groups using the chi-square test and Fisher's exact test. iPFS, PFS, and OS were evaluated by the Kaplan-Meier method. Survival time was compared using the log-rank test. Factors associated with survival were analyzed using Cox proportional hazards models in univariate and multivariate analyses, including covariates with p-values < 0.05 and variables associated with clinical outcomes. All statistical analyses were performed using SPSS 25.0 (IBM, Armonk, NY, USA) and GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA), $p < 0.02$ was considered statistically significant.

Results

Patients

A total of 113 driver gene mutation negative patients with NSCLC and BMs were enrolled (Fig. 1). They were divided into two groups: (1) 51 patients with PD-1 inhibitor monotherapy and 62 with PD-1 inhibitor plus anti-angiogenic therapy. Relevant clinical information of all patients was collected, covering demographic information, Eastern Cooperative Oncology Group Performance Status (ECOG-PS) score, pathological type, genetic mutation status, number and size of BMs, systemic and local treatment plans, and line number of previous treatment.

Baseline characteristics are shown (Table 1). On the whole, the two groups of patients had good balance and were comparable. No statistical significance was observed between the two cohorts in age ($p = 0.699$), gender ($p = 0.274$), smoking history ($p = 0.146$), ECOG-PS ($p = 0.395$) and histology ($p = 0.083$). Though the most common mutation in both groups was TP53, the specific mutation was unknown in the majority of patients. PD-L1 expression was negative in 18 patients (35.2%) and positive in 19 patients (37.3%) in the PD-1 inhibitor monotherapy group, while in the PD-1 inhibitor plus anti-angiogenesis therapy cohort, 17 patients (27.4%) PD-L1 expression were negative and 18 patients (29.1%) were positive, and 41 patients (36.2%) had no assessment of PD-L1 expression. Twenty patients (39.2%) in the PD-1 inhibitor monotherapy arm and 24 patients (38.7%) in the PD-1 inhibitor plus anti-angiogenesis therapy arm were defined at baseline by active BM on brain

Table 1. Basic characteristics of patients in the PD-1 inhibitor monotherapy and PD-1 inhibitor plus anti-angiogenesis therapy groups.

Characteristic, n (%)	PD-1 inhibitors monotherapy (n = 51)	PD-1 inhibitor plus anti-angiogenesis (n = 62)	Statistical value	*p* value
Age (years)			0.149	0.699[a]
Median	64	63		
Range	48–81	45–78		
<65	22 (43.1)	29 (46.8)		
≥65	29 (56.9)	33 (53.2)		
Gender			1.197	0.274[a]
Male	33 (64.7)	46 (74.2)		
Female	18 (35.3)	16 (25.8)		
Smoking history			2.110	0.146[a]
Former or active smoker	26 (51.0)	40 (64.5)		
Never smoker	25 (49.0)	22 (35.5)		
ECOG-PS			0.724	0.395[a]
0–1	35 (68.6)	47 (75.8)		
>1	16 (31.4)	15 (24.2)		
Histology			4.974	0.083[a]
Adenocarcinoma	32 (62.7)	43 (69.4)		
Squamous-cell carcinoma	10 (19.7)	16 (25.8)		
Other	9 (17.6)	3 (4.8)		
Mutation			3.279	0.680[b]
TP53	10 (19.6)	8 (12.9)		
KRAS	1 (2.0)	3 (4.8)		
BRAF	5 (9.8)	5 (8.1)		
ROS1	4 (7.8)	2 (3.2)		
WT	6 (11.8)	7 (11.3)		
Unknown	25 (49.0)	37 (59.7)		
PD-L1 expression			3.136	0.208[a]
Negative	18 (35.2)	17 (27.4)		
Positive	19 (37.3)	18 (29.1)		
Not evaluated	14 (27.5)	27 (43.5)		
Number of PD-1 inhibitors treatment lines			2.145	0.342[a]
1	20 (39.2)	28 (45.2)		
2	17 (33.3)	24 (38.7)		
≥3	14 (27.5)	10 (16.1)		
Steroid use			0.107	0.744[a]
NO	39 (76.5)	49 (79.0)		
YES	12 (23.5)	13 (21.0)		
Brain metastasis at diagnosis			0.139	0.709[a]
NO	24 (47.1)	27 (43.5)		
YES	27 (52.9)	35 (56.5)		
Active Brain metastasis			0.003	0.956[a]
NO	31 (60.8)	38 (61.3)		
YES	20 (39.2)	24 (38.7)		
Brain radiation therapy			10.928	0.010[b]
No local treatment	22 (43.1)	26 (41.9)		
SRS	8 (15.7)	21 (33.9)		
WBRT	20 (39.2)	10 (8.1)		
Surgery	1 (2.0)	5 (8.1)		
Number of BMs at 1st cycle of PD-1 inhibitors			0.380	0.538[a]
1–2	25 (49.0)	34 (54.8)		
>2	26 (51.0)	28 (45.2)		

Note: [a] compared by chi-square test; [b] compared by Fisher's exact test. ECOG, Eastern Cooperative Oncology Group; TP53, tumor protein p53; KRAS, Kirsten rat sarcoma viral oncogene homolog; BRAF, v-raf murine sarcoma viral oncogene homolog B1; ROS1, ROS Proto-Oncogene 1, Receptor Tyrosine Kinase; WT, wild type; SRS, stereotactic radiosurgery; WBRT, whole brain radiotherapy. $p < 0.02$ was considered statistically significant.

Table 2. Intracranial evaluation of curative effect.

Best Intracranial response	PD-1 inhibitors monotherapy n (%)	PD-1 inhibitor plus anti-angiogenesis therapy n (%)	Statistical value	*p* value
Complete response	1 (2.0)	5 (8.1)	1.037	0.220[b]
Partial response	11 (21.1)	18 (29.0)	0.817	0.366[a]
Stable disease	9 (17.3)	19 (30.6)	2.536	0.111[a]
Objective response rate	12 (23.1)	23 (37.1)	2.409	0.121[a]
Disease control rate	21 (40.4)	42 (67.7)	8.005	0.005[a]
Progressive disease	44 (84.6)	48 (77.4)	1.450	0.228[a]

Note: [a] compared by chi-square test; [b] compared by Fisher's exact test. $p < 0.02$ was considered statistically significant.

Table 3. Adverse events.

Adverse event	Any Grade				Grade ≥3			
	Monotherapy (n = 51)	Combined with anti-angiogenesis (n = 62)	Statistical value	*p*-value	Monotherapy (n = 51)	Combined with anti-angiogenesis (n = 62)	Statistical value	*p*-value
Rash	12 (23.5)	17 (27.4)	0.222	0.638[a]	1 (1.9)	2 (3.2)	0.000	1.000[b]
Thyroid dysfunction	25 (49.1)	29 (46.8)	0.057	0.812[a]	0 (0)	2 (3.2)	0.333	0.564[b]
Fatigue	10 (19.6)	19 (30.6)	1.787	0.181[a]	1 (1.9)	1 (1.6)	0.000	1.000[b]
Elevated liver enzymes	21 (41.2)	35 (56.4)	2.612	0.106[a]	0 (0)	0 (0)	0.000	1.000[b]
Abnormal renal function	4 (7.8)	2 (3.2)	0.446	0.407[b]	0 (0)	0 (0)	0.000	1.000[b]
Bellyache/Diarrhea	6 (11.7)	7 (11.2)	0.006	0.937[a]	0 (0)	0 (0)	0.000	1.000[b]
Oral mucositis	5 (9.8)	7 (11.2)	0.065	0.799[a]	0 (0)	0 (0)	0.000	1.000[b]
ICIs-related Pneumonia	2 (3.9)	5 (8.0)	0.267	0.605[b]	0 (0)	0 (0)	0.000	1.000[b]
Proteinuria	1 (1.9)	7 (11.2)	2.420	0.120[b]	0 (0)	0 (0)	0.000	1.000[b]
ICIs-related Myocarditis	0 (0)	1 (1.6)	0.000	1.000[b]	0 (0)	0 (0)	0.000	1.000[b]
Hypertension	2 (3.9)	8 (12.9)	1.796	0.180[b]	0 (0)	1 (1.6)	0.000	1.000[b]
Hand-foot syndrome	0 (0)	3 (4.8)	1.008	0.315[b]	0 (0)	1 (1.6)	0.000	1.000[b]

Note: [a] compared by chi-square test; [b] compared by Fisher's exact test. $p < 0.02$ was considered statistically significant.

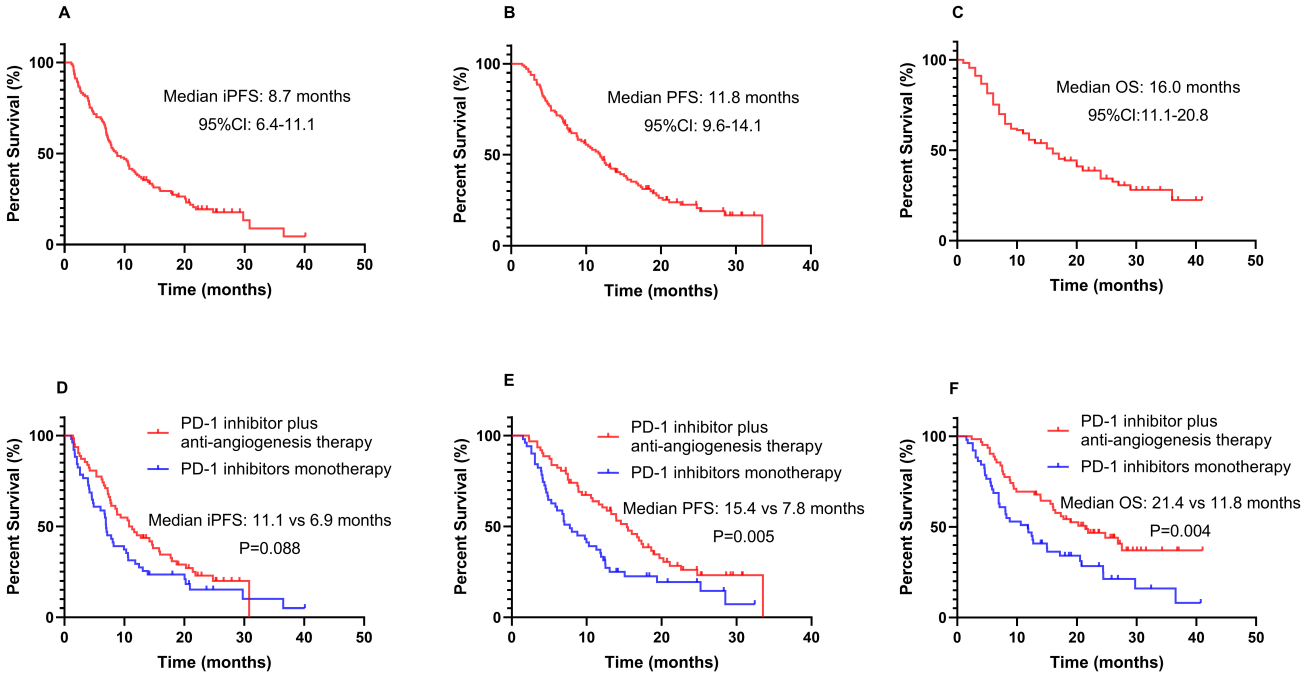

Fig. 2. Kaplan-Meier survival curves. Survival outcome of iPFS (A), PFS (B) and OS (C), in all BM patients (n = 113). (D) iPFS in the PD-1 inhibitor plus anti-angiogenesis therapy and PD-1 inhibitors monotherapy cohorts. (E) PFS in the PD-1 inhibitor plus anti-angiogenesis therapy and PD-1 inhibitors monotherapy cohorts. (F) OS in the PD-1 inhibitor plus anti-angiogenesis therapy and PD-1 inhibitors monotherapy cohorts. OS, overall survival; iPFS, intracranial progression-free survival; PFS, progression-free survival; BM, Brain metastases; CI, confidence interval.

MRI and clinical features. For different intracranial local treatment, the comparison between the two groups was statistically significant (p = 0.010). Most patients in the PD-1 monotherapy group chose radiotherapy as a local treatment. Eight patients (15.7%) had received SRS, 20 patients (39.2%) had been treated with whole brain radiation therapy (WBRT) and only one patient (2.0%) underwent surgery. In the PD-1 inhibitor plus anti-angiogenesis therapy group, 21 patients (33.9%) had received SRS, 10 patients (8.1%) had received WBRT and 5 patients underwent surgery. 25 patients had used steroids before or during PD-1 inhibitor therapy. Most patients were initiated with PD-1 inhibitors at the first or second lines.

Intracranial Efficacy

One patient in the PD-1 inhibitor monotherapy cohort achieved iCR, 11 patients obtained iPR, and 9 patients exhibited iSD, yielding an iORR of 23.1% and iDCR of 40.4% (Table 2). In the PD-1 inhibitor plus anti-angiogenesis therapy group, 5 patients achieved iCR, and 18 patients achieved iPR, 19 patients exhibited iSD, resulting in an iORR of 37.0% and iDCR of 67.7%. Until the final follow-up, 44 patients developed iPD in the PD-1 monotherapy cohort, while 48 patients in the PD-1 inhibitor plus anti-angiogenesis therapy cohort developed iPD. Though iDCR was significantly greater in the PD-1 plus anti-angiogenesis therapy cohort than in the PD-1 monotherapy cohort (p = 0.005), there was no significant difference between the two cohorts in iORR (p = 0.121).

Outcome of Patients

By January 2023, the median follow-up time was 26.2 months, and a total of 62 patients (67.4%) had confirmed iPD by craniocerebral MRI and RECIST1.1 criterion. Meanwhile, 30 patients were defined as iPD due to death; 86 of the 113 patients exhibited PD, 74 (65.5%) patients were verified to have died after follow-up. In all patients whose median OS was 16.0 months (95% confidence interval (CI): 11.1–20.8), median iPFS was 8.7 months (95% CI: 6.4–11.1), and median PFS was 11.8 months (95% CI: 9.6–14.1) (Fig. 2A–C). In comparison of survival time between the two groups, the anti-angiogenesis significantly improved patients' OS (21.4 *vs.* 11.8 months; p = 0.004), although no significant benefit was observed in iPFS (11.1 *vs.* 6.9 months; p = 0.088) (Fig. 2D–F); further subgroup analysis indicated that Anlotinib group (n = 20) (p = 0.011) improved the OS of patients (**Supplementary Fig. 1A–D**).

To analyze the benefit of PD-1 inhibitor plus anti-angiogenic therapy compared with PD-1 inhibitor monotherapy, we used Kaplan-Meier survival analysis and log-rank test. In terms of marginalization patients, there was no significant change in overall survival for patients with ECOG-PS 0-1 and ECOG-PS $\geq$2 treated with combination therapy or monotherapy (Fig. 3A,B, p = 0.050 and

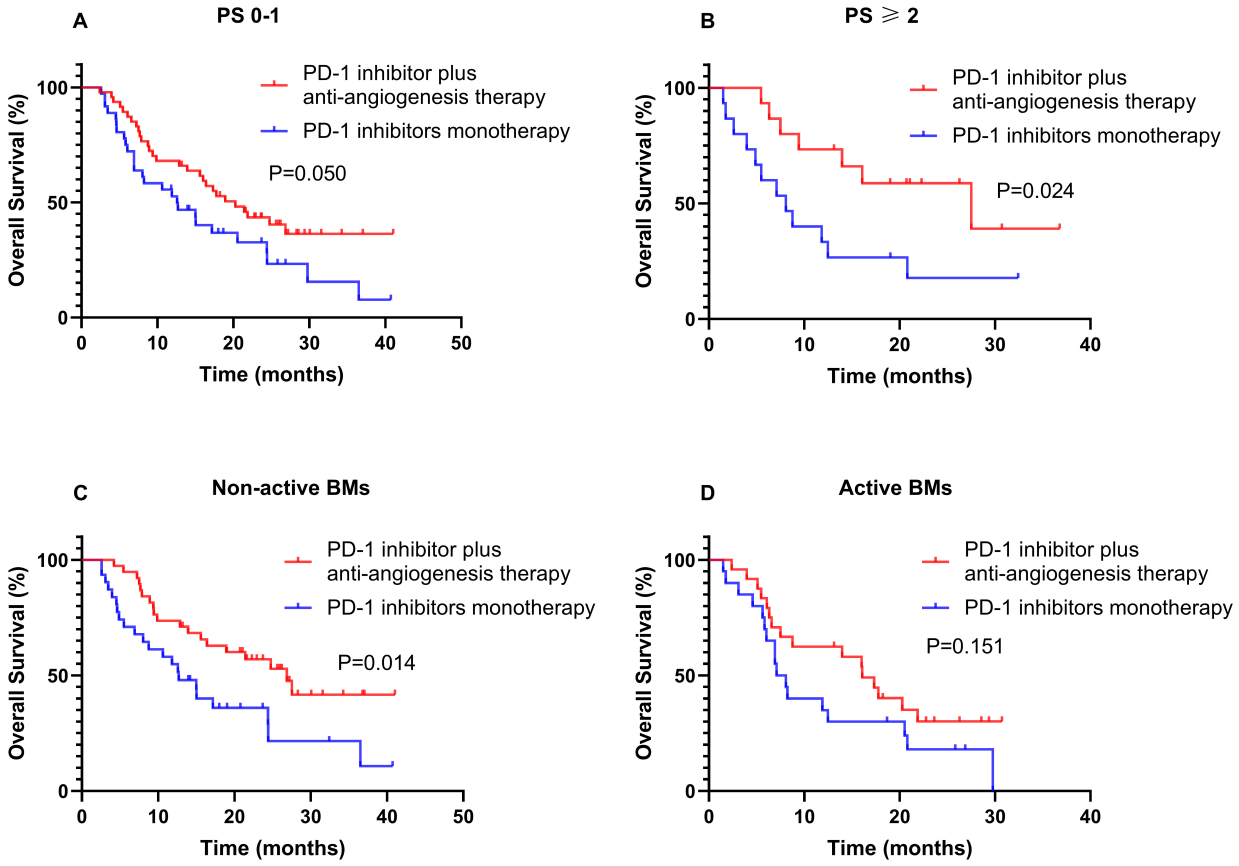

Fig. 3. Kaplan-Meier survival curves. (A) OS in PS 0–1 patients. (B) OS in PS ≥2 patients. (C) OS in patients with non-active BMs. (D) OS in patients with active BMs. BMs, Brain metastases; PS, Performance status.

p = 0.024). Both patients with and without active BMs benefit from PD-1 inhibitor plus anti-angiogenic therapy (Fig. 3C,D). Regarding therapeutic, patients with a history of local intracranial therapy (**Supplementary Fig. 2B**, p = 0.005) and no steroid therapy (**Supplementary Fig. 2C**, p = 0.015) may benefit more from the combination group. However, the overall survival of patients without a history of local intracranial therapy (**Supplementary Fig. 2A**, p = 0.262) and those with steroid therapy (**Supplementary Fig. 2D**, p = 0.140) did not differ significantly between the two therapy groups.

Safety

There was no statistically significant difference between the PD-1 inhibitor monotherapy and PD-1 inhibitor plus anti-angiogenesis therapy groups in AEs (Table 3). In the PD-1 inhibitor monotherapy group, the most common treatment-related AEs were thyroid dysfunction (49.1%), elevated liver enzymes (41.2%), rash (23.5%), fatigue (19.6%) and bellyache/diarrhea (11.7%). The most frequent treatment-related AEs in the PD-1 inhibitor plus anti-angiogenesis therapy group were elevated liver enzymes (56.4%), thyroid dysfunction (46.8%), fatigue (30.6%), rash (27.4%), bellyache/diarrhea (11.2%), and oral mucositis (11.2%), Proteinuria (11.2%). Grade 3–4 AEs occurred in 11.2% (7 of 62) of the PD-1 inhibitor plus anti-angiogenesis therapy group, and 3.9% (2 of 51) of the PD-1 inhibitor monotherapy cohort (p = 0.178). Adding anti-angiogenic therapy increased the incidence of hypertension and hand-foot syndrome. 5 (4.4%) patients (1 in monotherapy group, 4 in PD-1 inhibitor plus anti-angiogenesis therapy group) discontinuing treatment due to adverse reactions, and 18 (15.9%) patients (3 in monotherapy group, 15 in PD-1 inhibitor plus anti-angiogenesis therapy group) adjusting dose or changing therapeutic regimen. No major life-threatening AEs or treatment-related fatalities were observed in either group.

Univariate and Multivariate Analysis

Furthermore, diverse variables were also analyzed to determine whether any features were associated with survival outcome (Table 4). The results showed that the PD-1 inhibitors therapy regime (p = 0.003) and number of BMs at 1st cycle of PD-1 inhibitors (p = 0.001) were independent prognostic factors of OS. Likewise, multivariate analyses of PFS revealed that start PD-1 inhibitors early (first or second line) (p = 0.010) had longer PFS (**Supplementary Table 1**). Meanwhile, no statistically significant clinical factors were observed in the multivariate regression analysis of iPFS (**Supplementary Table 2**).

Table 4. Univariate and multivariate cox regression analysis of factors associated with overall survival.

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age (<65 *vs.* ≥65)	1.439	0.902–2.297	0.127	1.616	0.994–2.625	0.053
Gender (Female *vs.* Male)	1.196	0.721–1.984	0.489	0.886	0.366–2.149	0.789
Smoking history (Never *vs.* Former or active smoker)	1.298	0.810–2.079	0.278	1.582	0.692–3.617	0.277
Performance status (0–1 *vs.* >1)	1.067	0.633–1.799	0.807			
Squamous-cell carcinoma (NO *vs.* YES)	0.652	0.357–1.188	0.162			
PD-L1 expression						
Positive	reference	reference		reference	reference	
Negative	1.260	0.710–2.236	0.431	1.415	0.781–2.566	0.252
Not evaluated	1.065	0.612–1.851	0.824	1.191	0.673–2.110	0.548
PD-1 inhibitors line (≤2 *vs.* >2)	1.655	0.970–2.821	0.064			
Immunotherapy regimen (Monotherapy *vs.* Combination therapy)	0.512	0.323–0.810	0.004	0.483	0.299–0.780	0.003
Systemic steroid use (NO *vs.* YES)	1.648	0.967–2.810	0.066			
Brain local treatment (NO *vs.* YES)	1.200	0.755–1.907	0.440			
Active Brain Metastasis (NO *vs.* YES)	1.615	1.017–2.564	0.042	1.375	0.859–2.201	0.184
Number of BMs at 1st cycle of PD-1 inhibitors >2 (NO *vs.* YES)	2.386	1.488–3.826	0.001	2.234	1.379–3.617	0.001

$p < 0.02$ was considered statistically significant.

Discussion

Our research on driver gene mutation negative NSCLC and BMs assessed the efficacy and safety of PD-1 inhibitors with or without anti-angiogenic therapy, revealing that combination therapy significantly improves the OS of BMs patients. Additionally, PD-1 inhibitor plus anti-angiogenic treatment was well tolerated versus monotherapy. As a potentially more effective therapeutic regime, our data provide stronger evidence for subsequent clinical studies on NSCLC and BMs.

Given that the survival time of NSCLC BMs patients is shorter and due to other adverse clinical prognostic factors, most BMs patients have been excluded from vital immunotherapy studies for NSCLC [6,20–25]. Therefore, we can only estimate the efficacy of ICIs for NSCLC BMs patients in prospective single-arm studies of small samples, some large phase III randomized controlled studies of advanced NSCLC and real-world retrospective clinical data. In cohort 3 of the FIR study, 13 treated and asymptomatic NSCLC BM patients with PD-L1 secretion were enrolled, and the results showed that the ORR of the atezolizumab arm was 23%, the median PFS was 4.3 months, and the median OS was 6.8 months [9]. Additionally, in the Goldberg *et al.* [10] phase II clinical study, the iORR was 29.7% for 37 PD-L1-positive patients, and the median iPFS was 2.3 months and OS was 9.9 months. These results are consistent with our research. In the monotherapy group, the iORR was 23.1%, median PFS and OS were 7.8 months and 11.8 months, respectively. In general, it is not difficult to find intracranial efficacy of ICIs, but there are still some challenges. First, patients with active BMs were excluded. According to Tozuka [26], PD-1/PD-L1 monotherapy may be ineffective against active BMs, for which combination therapeutic regimes are necessary to evaluate those patients. Second, inclusion criteria were limited (good general condition was required). By comparison, our data indicate that PD-1 inhibitors + anti-angiogenic therapy benefits those with or without active BMs; The "disadvantaged groups" in actual clinical practice should not be directly ignored, and more consideration should be given to them to formulate a safe and effective treatment.

Depending on the mechanism of action, anti-angiogenic agents are divided into two categories: monoclonal antibodies (mAbs) targeting VEGF and multitarget tyrosine kinase inhibitors (TKIs). Bevacizumab, an anti-VEGF humanized mAb, binds to the vascular endothelial growth factor receptor (VEGFR) found in cancer cells and blocks the angiogenesis pathway, thereby reducing tumor vascular permeability and inhibiting tumor growth [27]. A phase III study evaluated nivolumab with bevacizumab and chemotherapy for non-squamous NSCLC, and for the BMs group compared to placebo. The nivolumab cohort showed an improved median PFS [28]. Similarly, in light of the IMpower150 study, the intracranial efficacy of ICIs combined with anti-angiogenesis can be elucidated [11,18]. Although these studies reveal important findings, there are limitations. Both include non-squamous NSCLC and exclude untreated or symptomatic BM. In contrast, in our enrolled NSCLC patients, no effect of non-squamous and squamous pathological types on survival outcome was observed.

Androtinib is a multi-target small-molecule anti-angiogenic drug, which has a wide range of inhibitory effects on tumor angiogenesis and growth [29]. A phase III ALTER0303 study showed that anlotinib has intracranial efficacy [30], and preclinical evidence also confirmed the effects of PD-1 blockade inhibitors plus anlotinib synergistic therapeutic [31,32]. Subsequently, Xiong *et al.* [33] and

Zhang *et al*. [34] retrospectively reported that immunotherapy combined with anlotinib had a benefit on PFS, but the specific analysis of BM patients was lacking. Wang *et al*. [35] showed support for PD-1 inhibitor plus anlotinib therapy, observing that the median PFS and OS of 16 (24%) NSCLC BMs patients were 5.8 months and 8.3 months, respectively. However, the basic characteristics and comparative effectiveness of BM patients have not been documented in detail [35]. In addition to complementing the deficiencies of the above research, our PD-1 inhibitors + anlotinib subgroup had longer survival. In addition, the small molecule multi-target TKI apatinib in combination with ICIs has also shown efficacy in animal models [36] and clinical studies [37]. Our results are encouraging and should be validated in a large cohort with BMs. More importantly, as stated in our results, OS is increased when combined with anlotinib, further confirming the anti-angiogenic effect in intracranial tissue [30,38]. In brief, in light of these findings, combining anti-angiogenesis therapy with ICIs should be considered an appropriate treatment options for patients with driver gene mutation negative NSCLC and BMs.

In our study, the AEs were similar between the two groups; grade 3–4 AEs were not significantly different, and neither cohort suffered a fatal adverse event. The most common AE in the monotherapy arm was thyroid dysfunction, consistent with a previous report [39]. Compared to monotherapy, the use of anti-angiogenic agents added extra AEs, such as hypertension and hand-foot syndrome, whose elevation was primarily graded as 1/2. Of note, one case of ICI-related myocarditis was found in the combination group. Despite being relatively rare, adverse cardiovascular effects are associated with severe consequences. In addition to the risk of ICI-related cardiac toxicity, NSCLC patients frequently have cardiac and cardiovascular risk factors (hypertension, dyslipidaemia, obesity) [40].

The study has several limitations. First, survival and selection biases were unavoidable due to the non-randomized and retrospective study design. Second, the sample size of this study is small, so the current results need to be interpreted with caution. Afterwards, for different gene mutations, the sample size was too small to be better stratified for further comparison, and patients with EGFR/ALK variant who might have benefited from ICI treatment were not included [18,41]. Additionally, a stratified analysis with a limited sample size may cause analytical errors due to the reduction of the sample size in each layer. However, even with some shortcomings, results from large randomized controlled phase III clinical trial in BMs patients have not been reported, suggesting that our research findings can be regarded as meaningful.

Conclusions

In conclusion, by comparing the combination therapy of PD-1 inhibitor and anti-angiogenic drugs with PD-1 inhibitor monotherapy in driver gene mutation negative NSCLC BMs patients, our results showed that the combination therapy is superior to monotherapy. In addition, we did not specifically select BMs patients to make them more representative. Nevertheless, there are still some issues that need to be solved before clinical application, such as the timing and duration of combination therapy and the dosage, to maximize the efficacy of combination therapy when it is safe and feasible. Simultaneously, a crucial concern for future research is to explore biomarkers related to the effect of ICIs on driver gene mutation negative NSCLC BMs patients. Finally, it is hoped that more prospective or multicentre retrospective studies with larger samples can verify and expand our research findings.

Abbreviations

NSCLC, Non–small-cell lung cancer; BM, Brain metastases; OS, Overall survival; PFS, Progression-free survival; iPFS, Intracranial Progression-free survival; iDCR, Intracranial disease control rate; iORR, Intracerebral objective response rate; HR, Hazard ratio; CI, Confidence interval; AEs, Adverse events; ICIs, Immune checkpoint inhibitors; VEGF, vascular endothelial growth factor; mAbs, Monoclonal antibodies; TKIs, Tyrosine kinase inhibitors; ECOG, Eastern Cooperative Oncology Group.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

JQS, CYZ and AWL designed the research study. JQS, CYZ and AWL performed the research. XW, ZMZ and PAL provided help and advice on experiments. XW, ZMZ and PAL analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This retrospective study of patients who were not randomized to treatment groups was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Second Affiliated Hospital of Nanchang University and Jiangxi Provincial People's Hospital, The First Affiliated Hospital of Nanchang Medical College ([2022] No. 007). Informed consent was exempted because of the retrospective nature of this study.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202335176.33.

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