Supplementary materials

Content

Methods

Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for this trial.

1. Aged 18 to 75, male or female;
2. Moderately differentiated or undifferentiated locally recurrent/metastatic nasopharyngeal carcinoma (WHO class II-III) in histopathology;
3. Patients in clinical stage IVb who have failed first-line platinum-based monotherapy or combined chemotherapy and second-line monotherapy or combined chemotherapy (defined according to the Chinese 2008 staging system for nasopharyngeal carcinoma 2017 edition). Definition of treatment failure: Progression during or after chemotherapy following recurrence/metastasis; concurrent chemoradiotherapy, with progression within 6 months, may be counted as first-line treatment. All changes of regimen due to drug intolerance are not considered treatment failure;
4. ECOG PS: 0-1;
5. Expected survival ≥ 12 weeks;
6. At least one measurable lesion per the Response Evaluation Criteria in Solid Tumors (RECIST 1.1), and the measurable lesions should not have been treated locally such as with radiotherapy;
7. Fresh tissues or tissue samples for biomarker (such as PD-L1) analysis must be provided. (Fresh tissues are preferred. Archived samples of 5-8 paraffin embedded sections with a thickness of 3-5 μm are also acceptable if a fresh biopsy is not accessible)
8. Major organ functions must meet the following requirements (No blood components or cell growth factors are allowed within 2 weeks prior to the start of study treatment):
    a. Absolute neutrophil count (ANC) ≥ 1.5×10^9/L
    b. Platelets (PLT) ≥ 90×10^9/L;
    c. Hemoglobin (Hb) ≥ 9 g/L;
    d. Serum albumin ≥ 2.8 g/dL;
    e. Bilirubin ≤ 1.5 × ULN, ALT and AST ≤ 1.5 × ULN; for liver metastasis, ALT and AST ≤ 5 × ULN;
    f. CrCl ≥ 50 mL/min (Cockcroft-Gault);
9. Female subjects of childbearing age must have a negative pregnancy test result within 72 h prior to the start of study treatment, and be willing to take at least 2 highly effective contraceptive measures during the course of the trial and 60 days after the last dose of the investigational product. Male subjects with partners of childbearing potential must take at least two contraceptive measures during the course of the trial and 120 days after the last dose of the investigational product;

10. Subject must participate voluntarily, sign the informed consent form, have good compliance, and cooperate with follow-up visits.

Exclusion Criteria

Subjects meeting any of the following criteria must be excluded:

1. Patients with any active autoimmune disease or a history of autoimmune disease (e.g., interstitial pneumonia, uveitis, enteritis, hepatitis, hypophysitis, vasculitis, myocarditis, nephritis, hyperthyroidism, and hypothyroidism (may be enrolled after effective hormone replacement therapy); patients with vitiligo or asthma in childhood that has completely relieved and requires no intervention in adulthood and patients requiring medical interventions with bronchodilators may be enrolled);
2. Patients with clinically symptomatic metastases to central nervous system (e.g., cerebral edema requiring hormone interventions, or progression of brain metastasis). Patients with previously treated brain metastases were eligible if they were clinically stable (without the need of prednisone of more than 10 mg/day or equivalent hormone therapy);
3. Patients with a history of or currently active malignant tumors (except for malignant tumors that have been cured with a cancer-free survival of more than 5 years, e.g., basal cell carcinoma, cervical carcinoma *in situ*, and papillary thyroid carcinoma);
4. Patients with uncontrolled cardiac symptoms or disease, such as (1) NYHA Class II or higher cardiac failure, (2) unstable angina, (3) myocardial infarction within the past year, and (4) clinically significant supraventricular or ventricular arrhythmias requiring clinical interventions;
5. Patients requiring systemic treatment with corticosteroids (> 10 mg/day of prednisone or equivalent) or other immunosuppressive medications within 14 days prior to administration of the investigational product. In the absence of active autoimmune disease, inhaled or topical use of corticosteroids and an equivalent dose to > 10 mg/day of prednisone for adrenal hormone replacement are permitted;
6. Patients who have received chemotherapy and targeted therapy within 4 weeks prior to the study treatment; palliative radiotherapy for symptomatic control is permitted but must be completed at least 2 weeks prior to the start of study treatment, and no additional radiotherapy should be scheduled for the same lesion; patients with an AE induced by prior treatment that has not resolved to CTCAE Grade ≤ 1 (except for alopecia and sequelae of relevant neurotoxicity of previous platinum therapy);
7. Patients with active infection or unexplained pyrexia of > 38.5 °C at screening or prior to the first dose (those with tumor-induced pyrexia may be enrolled as per the judgment of the investigator);

8. Congenital or acquired immune deficiency (such as HIV infection), active hepatitis B (HBV-DNA ≥ 10^4 copies/mL or 2000 IU/mL) or hepatitis C (positive anti-HCV antibodies, and HCV RNA titer higher than the lower limit of detection of the analytical method);
9. Patients who have participated in other clinical studies within 1 month before the start of study treatment;
10. Patients who have received live vaccines within 4 weeks before the start of study treatment;
11. Patients who have used systemic antibiotics within 1 month before the start of study treatment;
12. Patients who have received previous treatment with other anti-PD-1 antibodies or other checkpoint monoclonal antibodies, including immunotherapy targeting CTLA-4 and PD-L1;
13. Patients with known history of psychotropic substance abuse, alcoholism, or drug abuse;
14. Pregnant or lactating women;
15. Other factors, as determined by the investigator, which may result in premature discontinuation of treatment.

Biomarker analysis

Archived or fresh tumor samples were retrieved for immunohistochemical staining of PD-L1 expression in tumor cells with SP142 antibody (Abcam, Cambridge, MA, USA). PD-L1 expression was scored as the percentage of tumor cells with membranous staining and thresholds of 1%, 10%, and 20% were used for subgroup analysis.

Multiplex immunofluorescence staining analysis of immune cell population with staining of multiple immune-related biomarkers were also conducted, including MHC-I (clone EMR8-5), MHC-II (clone CR3/43), CD68 (clone KP1), CD86 (clone EP1158-37), Granzyme B (clone EPR8260), Granzyme H (clone CSP-C), CD8A (clone EP1150), IFN-γ (clone EPR21704), FoxP3 (clone SP97), CD19 (clone EPR5906), and CD4 (clone SP35). CK-Pan (clone PAN-CK) was used as epithelial cell marker. All antibodies were from Abcam (Cambridge, MA, USA). Expression of immune-related biomarkers were scored by cell density, which was defined as numbers of cells with positive protein expression within tumor and stroma per mm^2. The signals were acquired with digital slice scanner (KF-PRO-020, KFBIO, Ningbo, China), and analyzed using HALO (Indica Labs, NM, USA).

Endpoints

The primary endpoint was independent review committee-assessed objective response rate (ORR), which was defined as the proportion of patients who achieved complete response or partial response per Response Evaluation Criteria in Solid tumors version 1.1 (RECIST 1.1). The secondary endpoints were investigator-assessed ORR, duration of response (time from

first documented objective response to disease progression or death from any cause, whichever occurred first), disease control rate (the proportion of patients who had objective response or stable disease), time to response (time from study drug administration to onset of response), progression-free survival (time from study drug administration to disease progression per RECIST 1.1 or death from any cause, whichever happened first), and overall survival (time from study drug administration to death).

Table 1. The antitumor activity in patients with different PD-L1 expression level per independent review committee

	PD-L1 expression					
Activity	**≥1% (n=114)**	**<1% (n=36)**	**≥10% (n=88)**	**<10% (n=62)**	**≥20% (n=68)**	**<20% (n=82)**
ORR, n (%) [95% CI]	34 (29.8) [21.6-39.1]	9 (25.0) [12.1-42.2]	31 (35.2) [25.3-46.1]	12 (19.4) [10.4-31.4]	24 (35.3) [24.1-47.8]	19 (23.2) [14.6-33.8]
PFS, median (95% CI), months	3.7 (2.0-4.8)	3.8 (1.8-5.4)	3.9 (2.7-7.8)	2.8 (1.8-3.8)	4.7 (2.7-9.4)	2.8 (1.9-3.8)
OS, median (95% CI), months	18.7 (16.0-22.5)	13.5 (8.3-22.0)	19.9 (17.5-NR)	14.2 (10.6-17.5)	19.9 (17.6-NR)	14.2 (10.9-17.5)

Abbreviations: PD-L1, programmed cell death ligand 1; ORR, objective response rate; PFS, progress-free survival; OS, overall survival; CI, confidence interval; NR, not reached.

Table 2. The survival in patient subgroups defined by a composite variable of PD-L1 expression (≥10% vs <10%) and density of MHC-II+ cells in stroma (above vs below 75th percentile)

Survival	MHC-II high PD-L1 high (n=15)	MHC-II low PD-L1 high (n=57)	MHC-II high PD-L1 low (n=15)	MHC-II low PD-L1 low (n=34)
PFS, median (95% CI), months	12.8 (3.7-NR)	3.6 (1.9-5.7)	3.9 (1.8-NR)	1.9 (1.8-3.7)
OS, median (95% CI), months	NR (15.7-NR)	18.7 (15.2-21.8)	15.0 (8.1-NR)	11.8 (8.0-17.5)

Abbreviations: PD-L1, programmed cell death ligand 1; MHC-II, major histocompatibility complex class II; PFS, progress-free survival; OS, overall survival; CI, confidence interval; NR, not reached.

Table 3. The antitumor activity in patients with baseline positive and negative plasma EBV DNA level per independent review committee

Activity	**Plasma EBV DNA level**	
	Positive (n=117)	**Negative (n=39)**
ORR, n (%) [95% CI]	28 (23.9) [16.5-32.7]	16 (41.0) [25.6-57.9]
DCB, n (%) [95% CI]	44 (37.6) [28.8-46.4]	20 (51.3) [35.6-67.0]
PFS, median (95% CI), months	2.7 (1.9-3.9)	6.0 (2.9-11.1)
OS, median (95% CI), months	16.5 (13.5-19.5)	22.7 (15.2-NR)

Abbreviations: EBV, Epstein-Barr virus; ORR, objective response rate; DCB, durable clinical benefit; PFS, progression-free survival; OS, overall survival, NR, not reached.

Table 4. The clinical outcomes in patients with ≥50% and <50% EBV DNA level decrease from baseline to the first post baseline assessment per independent review committee

	Plasma EBV DNA level decrease from baseline to the first post baseline assessment	
Activity	**<50% (n=59)**	**≥50% (n=56)**
ORR, n (%) [95% CI]	4 (6.8) [1.9-16.5]	24 (42.9) [29.7-56.8]
PFS, median (95% CI), months	1.9 (1.8-2.1)	5.5 (3.6-9.1)

Abbreviations: EBV, Epstein-Barr virus; ORR, objective response rate; PFS, progression-free survival.

Figure 1. Study profile

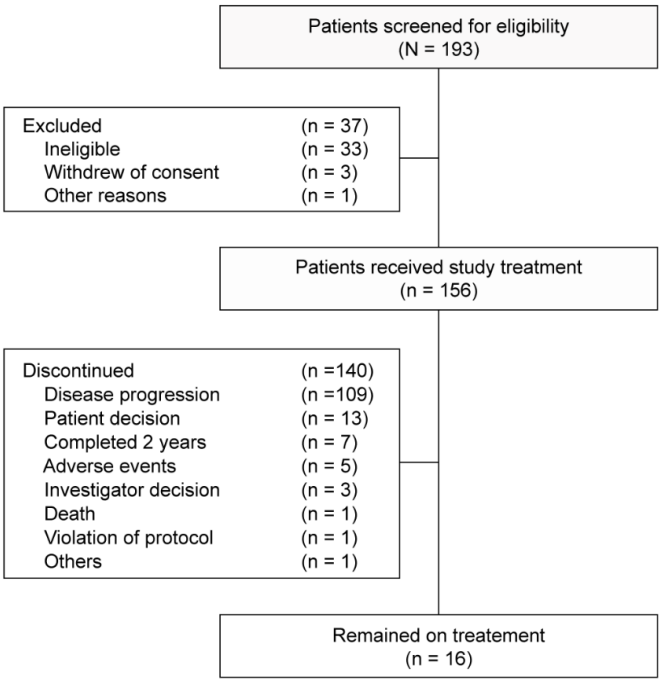
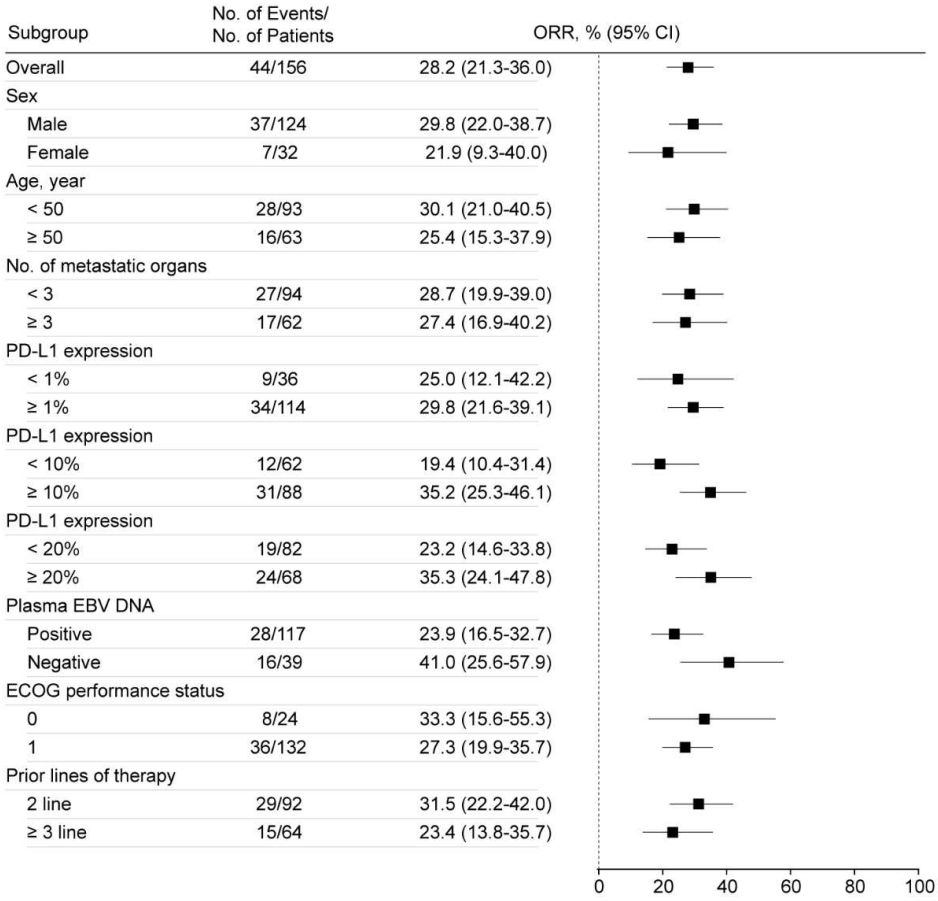
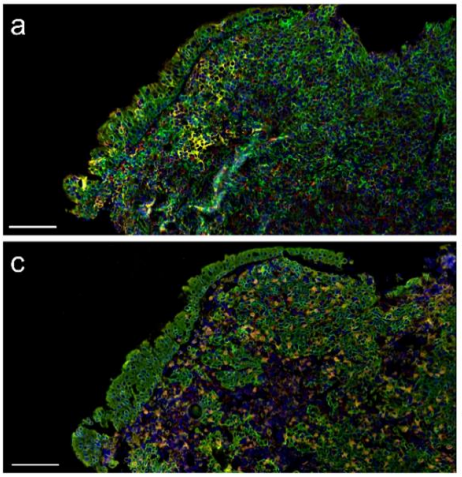


Figure 2. Subgroup analysis of ORR per independent review committee



Abbreviations: ORR, objective response rate; CI, confidence interval; PD-L1, programmed cell death ligand 1; EBV DNA, Epstein-Barr virus; ECOG, Eastern Cooperative Oncology Group.

Figure 3. Representative images of multiplex immunofluorescence staining of immune-related biomarkers



	Green	Orange	Red	Yellow
Panel 1	MHC-I	MHC-II	CD68	CD86
Panel 2	GZMB	GZMH	CD8A	IFN-γ
Panel 3	CK-Pan	FoxP3	CD19	CD4

a Panel 1. **b** Panel 2. **c** Panel 3

Abbreviations: GZMB, Granzyme B; GZMH, Granzyme H

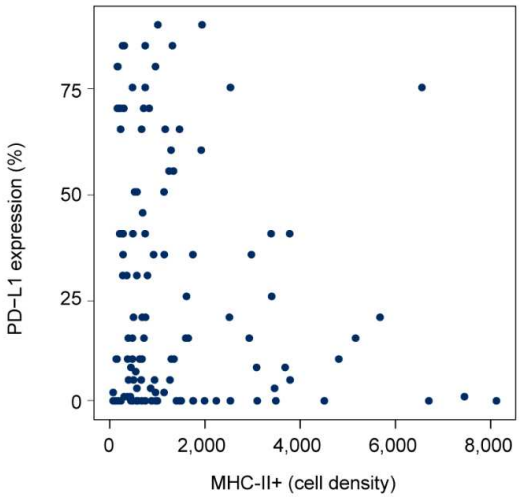
Bars represent 100 µm.

Figure 4. Correlation analysis of biomarker cell density with clinical benefit

a Correlation of biomarker cell density with objective response. **b** Correlation of biomarker cell density with durable clinical benefit.

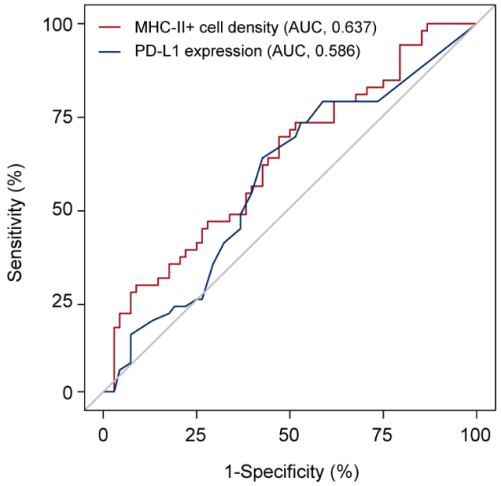
Numbers are *P* values, which were calculated using the Point-Biserial correlation analysis. The color red and blue represent positive and negative correlation. The circle size represents the correlation coefficient, which was calculated with -log10 (*P* value).

Figure 5. Scatter plot of PD-L1 expression versus MHC-II+ cell density



Abbreviations: MHC-II, major histocompatibility complex class II; PD-L1, programmed cell death ligand-1

Figure 6. Receiver operating characteristic curve analysis of PD-L1 expression and MHC-II+ cell density for durable clinical benefit



Abbreviations: MHC-II, major histocompatibility complex class II; PD-L1, programmed cell death ligand-1; AUC, area under the curve