A Phase II Study of Nivolumab plus Gemcitabine in Patients with Recurrent or Metastatic Nasopharyngeal Carcinoma (KCSG HN17–11)

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ABSTRACT

Purpose: Although programmed death 1/programmed death ligand 1 (PD-1/PD-L1) inhibitors are promising agents for recurrent or metastatic nasopharyngeal carcinoma (NPC), PD-1/PD-L1 inhibitor monotherapy has shown modest efficacy. This study evaluated the efficacy and safety of nivolumab plus gemcitabine in patients with NPC who failed prior platinum-based chemotherapy.

Patients and Methods: This is a phase II, multicenter, open-label, single-arm study. Patients with recurrent or metastatic NPC received nivolumab 3 mg/kg and gemcitabine 1,250 mg/m^2 every 2 weeks until disease progression or intolerable toxicity. The primary endpoint was progression-free survival (PFS). The secondary endpoints included objective response rate (ORR), overall survival (OS), and safety. To identify potential biomarkers, whole-exome sequencing, whole-transcriptome sequencing, and immune phenotype analysis based on Lunit SCOPE IO, an artificial intelligence–powered spatial tumor-infiltrating lymphocyte analyzer, were performed.

Results: Thirty-six patients were enrolled between June 2018 and June 2019. The ORR was 36.1% and disease control rate was 97.2%. With median follow-up of 22.0 months, median PFS was 13.8 months [95% confidence interval (CI), 8.6–16.8 months]. Median OS was not reached, and OS rate at 6 months was 97.0% (95% CI, 80.4%–99.6%). The grade ≥3 treatment-related adverse events were hypertension (2.8%) and anemia (2.8%). In multivariate analysis of mutation of chromatin modifier gene, tumor mutational burden (≥ 2.1 mut/Mb), and somatic copy-number alteration (SCNA) level, the group with high SCNA (> 3 points; HR, 7.0; 95% CI, 1.3–37.9; $P = 0.02$) had independently associated with poor PFS. Immune phenotype analysis showed that tumors with high proportion of immune-excluded immune phenotype was significantly correlated with poor PFS (HR, 4.4; 95% CI, 1.2–16.2; $P = 0.018$).

Conclusions: Nivolumab plus gemcitabine showed promising efficacy with favorable toxicity profiles in patients with advanced NPC in whom platinum-based combination chemotherapy failed.

Introduction

Nasopharyngeal carcinoma (NPC) has ethnic and geographic distributions and is common, especially in Southeast Asia and Northern Africa (1). NPC is associated with Epstein–Barr virus (EBV) infection. The programmed death 1/programmed death ligand 1 (PD-1/PD-L1) pathway has been suggested to play an important role in T-cell tolerance to EBV and tumor immune escape in NPC.

EBV-induced latent membrane protein 1 and IFNγ pathways cooperate to regulate PD-L1 (2). PD-L1 expression was detected in 95% of tumor cells. High expression of PD-L1 (median H-score > 35) in tumor cells significantly correlated with a poor prognosis of progression-free survival (PFS; ref. 3).

Once recurrence or metastasis occurs, the prognosis of NPC remains poor. Platinum-based combination chemotherapy is the standard treatment for patients with recurrent or metastatic NPC (4, 5). After progression to platinum-based combination chemotherapy, treatment options are limited.

Recently, PD-1/PD-L1 inhibitor monotherapy has shown a modest response and survival benefit in patients with recurrent or metastatic NPC who progressed to platinum-based combination chemotherapy (6, 7). Gemcitabine is an active and tolerable agent for platinum-failed NPC. Objective response rate (ORR) was 34% and PFS was 5.1 months (8). Moreover, gemcitabine reduces the frequency of CD11b+GR1+ myeloid suppressor cells (9). Gemcitabine-induced apoptosis of established tumor cells may enhance the dendritic cell–dependent cross-presentation of tumor antigens to T cells. Gemcitabine functions synergistically with CD40 stimulation of T cells. Hence, theoretically, gemcitabine may have a synergistic effect with the PD-1/PD-L1 blocking agent.

On the basis of this evidence, we evaluated the efficacy and safety of nivolumab and gemcitabine in recurrent or metastatic NPC that progressed to platinum-based combination chemotherapy.

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Translational Relevance

This study evaluated the efficacy and safety of nivolumab plus gemcitabine in patients with nasopharyngeal carcinoma (NPC) who failed prior platinum-based chemotherapy. Nivolumab plus gemcitabine showed 36.1% of objective response rate and 13.8 months of median progression-free survival (PFS). Patients with high somatic copy-number alteration (SCNA > 3) had poor PFS compared with patients with low SCNA (≤ 3). Immune phenotype analysis showed tumor with a high proportion of immune-excluded immune phenotype was correlated with poor PFS. Nivolumab and gemcitabine showed promising efficacy with favorable toxicity profiles in patients with platinum-failed NPC.

Patients and Methods

Participants

Patients who were diagnosed with histologically confirmed NPC and previously received at least one platinum-based cytotoxic chemotherapy for recurred or metastatic disease were eligible. Other key eligibility criteria included patients who had at least one measurable lesion based on RECIST 1.1 and had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. Patients with previously treated and radiologically stable brain metastasis could be enrolled. All patients provided written informed consent, and the study was reviewed and approved by the Institutional Review Board of each institute. This study was conducted in accordance with the Declaration of Helsinki (Clinical Trial identifier no. KCT0003189).

Study design and treatment schedule

This was an open-label, single-arm, phase II study of nivolumab plus gemcitabine. This study was conducted at seven affiliated institutes in the Korean Clinical Study Group (KCSG). Patients received nivolumab (3 mg/kg) and gemcitabine (1,250 mg/m^2) through intravenous infusion every 2 weeks. The cycle was repeated every 28 days. Patients received nivolumab and gemcitabine for up to 1 year or until disease progression, unacceptable toxicity, death, or withdrawal of consent. The continuation of treatment beyond progression was allowed according to the physician's judgment.

Response evaluation was performed on the basis of RECIST 1.1 using CT or MRI scan, every 8 weeks from the date of initiation of treatment. The safety objectives were evaluated according to the NCI Common Terminology Criteria, version 4.3. The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (QLQ-C30) was administered at baseline and weeks 8, 12, 18, and 24 (10).

Study endpoints

The primary endpoint of this study was PFS, and the secondary endpoints were the ORR, disease control rate, overall survival (OS), and safety. ORR was defined as the proportion of patients with complete response (CR) or partial response (PR), and disease control rate was defined as CR+ PR+ stable disease (SD) using RECIST criteria version 1.1. The relationship between the outcomes and potential biomarker were evaluated through whole-exome sequencing (WES) and whole-transcriptome sequencing (WTS).

Statistical analysis

Considering the median PFS of 5.1 months in patients with NPC who received second-line chemotherapy (8), we expected the median PFS of the experimental arm to be 8.0 months, an accrual time of 24 months, and a follow-up period of 12 months. A total of 36 patients was calculated with the power of 80% by one-sample log-rank test (one-sided alpha of 20%), and considering 10% dropout rate. PFS was the interval between the first date of the treatment and the date of disease progression or all-cause mortality. OS was the interval between the first date of the treatment and the date of all-cause mortality. We assessed QLQ-C30 at weeks 8, 12, 24, and 36. We described the change of mean score from baseline to follow-up weeks. All P values were two-sided, and a P value < 0.05 was significant. Data were analyzed using SAS version 9.4 (SAS Institute, Cary, NC). The data cutoff was April 30, 2021.

Next-generation sequencing

For WES, DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tissue and blood using Qiagen extraction kit.

Table 1. Baseline characteristics.

Patient characteristics	Total (N = 36)
Age (year), median (range)	50.5 years (20–81)
<60 years	25 (69.4%)
≥60 years	11 (30.6%)
Sex	
Male	30 (83.3%)
Female	6 (16.7%)
ECOG performance status	
0	3 (8.3%)
1	33 (91.7%)
Histologic diagnosis (WHO class), *n* (%)	
Keratinizing squamous cell carcinoma	1 (2.8%)
Non-keratinizing differentiated carcinoma	13 (36.1%)
Non-keratinizing undifferentiated carcinoma	14 (38.9%)
Others	8 (22.2%)
AJCC stage (8th edition) at initial diagnosis, *n* (%)	
I	0 (0.0%)
II	2 (5.6%)
III	9 (25.0%)
IVA	10 (27.8%)
IVB	11 (30.6%)
Unknown	4 (11.1%)
EBV *in situ* hybridization, *n* (%)	
Positive	26 (72.2%)
Negative	3 (8.3%)
Unknown	7 (19.4%)
Distant metastasis	
Lung	14 (38.9%)
Liver	11 (30.6%)
Bone	14 (38.9%)
Brain	0 (0.0%)
Others	13 (36.1%)
First treatment for NPC	
Chemoradiotherapy	29 (80.5%)
Palliative chemotherapy	7 (19.4%)
Line of previous palliative chemotherapy	
1	25 (69.4%)
2	4 (11.1%)
3	3 (8.3%)

Abbreviations: AJCC, American Joint Committee on Cancer; WHO, World Health Organization.

The specimen was small punched biopsy, so we did not perform microdissection. Raw sequencing reads were aligned to the hg19 reference genome using BWA-MEM, and GATK base quality score recalibration, indel realignment, and duplicated removal processing according to the GATK Best Practice recommendation were performed (11–13).

For WTS, total RNA was extracted from FFPE tissues using the Qiagen RNeasy FFPE kit, and purified RNA was used to generate libraries with the TruSeq RNA Access Library Preparation Kit. RNA reads were aligned to the hg19 reference genome using STAR v2.5.0a. Count values were normalized with RSEM algorithms.

Variant calling and copy-number alteration

We used Mutect2 and Manta-Strelka combinations to identify somatic single-nucleotide variants and insertions, or deletions (indels). We performed the gistic2 program with default options to find recurrent copy-number change regions in focal or broad regions (chromosome arm levels) using copy-number segments by CNV kit's result. The somatic copy-number alteration (SCNA) level was calculated as the sum of the absolute values of the genetic results by the broad or focal region.

Estimate of immune scores and related pathway analysis

To investigate immune-related features of NPC samples, we normalize the raw count using EdgeR's TMM method and extracted a list of differentially expressed genes to analyze gene set enrichment analysis (GSEA) using various annotated gene sets (Molecular Signature Database). We conducted to stromal score, immune score, and tumor purity for each sample using the "ESTIMATE" R software package.

Immune phenotype analysis by Lunit SCOPE IO

Lunit SCOPE IO was trained and validated with a 13.5×10^9 μm^2 area and 6.2×10^6 tumor-infiltrating lymphocytes (TIL) from 17,849 hematoxylin and eosin (H&E) whole slide images (WSI) of multiple (>16) cancer types including NPC, annotated by 104 board-certified pathologists (Supplementary Information). Immune phenotype was defined as follows (14, 15): inflamed as TIL density in the cancer area above the threshold ($200/mm^2$); immune-excluded as TIL density in the cancer area below the threshold and TIL density in the cancer stroma area above the threshold ($200/mm^2$); and immune-desert as both TIL density in the cancer area and that in the cancer stroma area below the thresholds. Inflamed, immune-excluded, and immune-desert scores of WSI were defined by the number of grids annotated to a certain immune phenotype divided by the total number of grids analyzed in the WSI.

Data availability statement

The data generated in this study are available within the article and its Supplementary Data file. Additional data are available upon request from the corresponding author.

Results

Study population and clinical characteristics

Between June 2018 and June 2019, 40 patients were screened. Two patients did not meet the inclusion criteria, and two withdrew consent before starting nivolumab plus gemcitabine treatment (Supplementary Fig. S1). A total of 36 patients were enrolled and received at least one treatment cycle. Therefore, 36 patients were included in the tumor response evaluation, safety evaluation, and survival analysis. **Table 1** shows the baseline patient characteristics. Median follow-up was 22.0 months (range 1.8–33.6). None of the patient received gemcitabine plus platinum as palliative first line, because gemcitabine plus platinum had not been approved in Korea when the study was initiated.

ORR and survival

The ORR was 36.1% (3 CRs and 10 PRs) and disease control rate was 97.2% (**Fig. 1A**; **Table 2**). **Figure 1B** shows the treatment duration and the best response by swimmer plot. Eight patients received nivolumab plus gemcitabine treatment beyond progression at the physician's discretion. Median PFS was 13.8 months [95% confidence interval (CI), 8.6–16.8 months; **Fig. 2A**]. The PFS rate at 6 and 12 months were 70.3% (95% CI, 51.8%–82.8%) and 60.6% (95% CI, 41.8%–74.9%), respectively. The median OS was not reached (**Fig. 2B**). The OS rates at 6 and 12 months were 97.0% (95% CI, 80.4%–99.6%) and 86.7% (95% CI, 70.4%–95.2%), respectively.

Safety profile

Serious adverse events were 4 (1 of fever, 1 of urinary tract obstruction, 2 of pneumonia), but none of them was related to the study treatment and all recovered without sequalae. **Table 3**

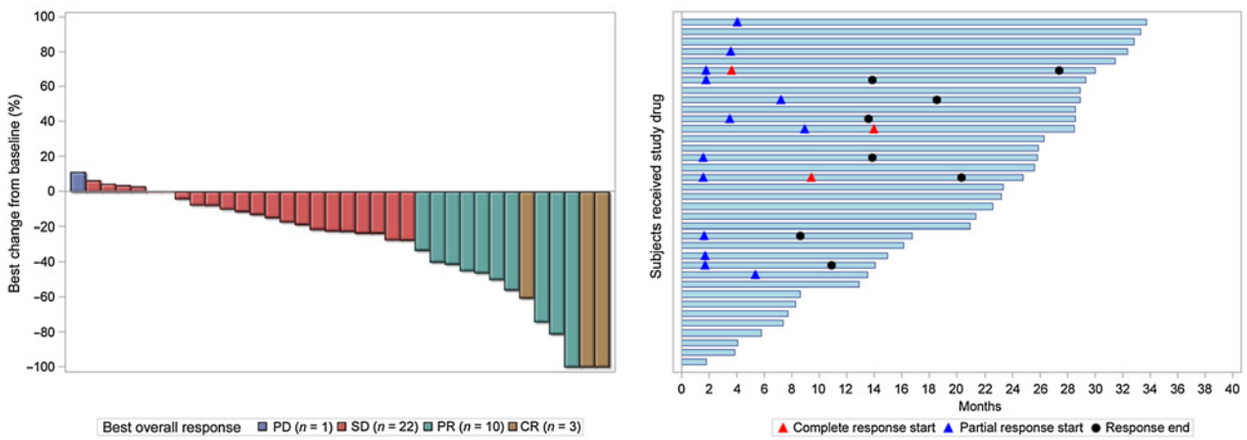

Figure 1.

A, Waterfall and (**B**) Swimmer plot of gemcitabine and nivolumab.

Table 2. Treatment response and survival outcomes to nivolumab plus gemcitabine.

Outcomes	Total ($N = 36$)
Best response, *n* (%)	
CR	3 (8.3%)
PR	10 (27.8%)
SD	22 (61.1%)
PD	1 (2.8%)
ORR, % (95% CI)	36.1% (20.4%–51.8%)
Disease control rate, % (95% CI)	97.2% (91.9%–102.6%)
Proportion PFS at 6 months, % (95% CI)	70.3% (51.8%–82.8%)
Proportion PFS at 12 months, % (95% CI)	60.5% (41.8%–74.9%)
Proportion OS at 6 months, % (95% CI)	97.0% (80.4%–99.6%)
Proportion OS at 12 months, % (95% CI)	87.7% (70.4%–95.2%)
No. of cycles, median (range)	12.9 (2–15)

Abbreviation: PD, progressive disease.

summarizes the adverse events per patients. The common treatment-related adverse events of any grade were nausea ($n = 8$; 22.2%), anorexia ($n = 6$; 16.7%), acneiform rash ($n = 6$; 16.7%), and fatigue ($n = 5$; 13.9%). The grade ≥3 treatment-related adverse events included hypertension ($n = 1$; 2.8%) and anemia ($n = 1$; 2.8%). At least one dose interruption occurred in 6 patients (16.7%) and dose reduction to 1,000 mg/m^2 of gemcitabine occurred in 1 patient (2.8%) and 750 mg/m^2 of gemcitabine in 1 patient (2.8%), primarily due to adverse events such as anemia, edema, and general weakness. Two patients (5.6%) stopped permanently due to toxicity of gemcitabine, but they continued nivolumab. The median dose administered was 1.49 mg/kg/week (range, 1.17–1.59) of nivolumab and 618.2 mg/m^2/week (range, 433.5–646.0) for gemcitabine.

Quality-of-life assessment

At baseline, 36 patients were compliant with QLQ-C30; at weeks 8, 12, 18, 24, 36 (100%) of 36; 32 (88.9%) of 36; 28 (77.8%) of 36; and 25 (69.4%) of 36 patients were compliant, respectively (Supplementary Table S1). From baseline to week 18, the global health status/quality of life (QOL) score showed a tendency to improve (mean score 6.05 points increased). From baseline to week 24, the global health status/QOL score slightly decreased (mean score 1.97 points decreased), but in general, it was maintained ($P = 0.86$). Functional scales, such as physical functioning, role functioning, emotional functioning, cognitive functioning, and social functioning, were maintained from baseline to week 24. The symptom scale score did not deteriorate from baseline to week 24.

Identification of somatic mutation in NPC

Among the 36 patients in this study, WES and WTS were performed in 22 patients. We found that gene set of chromatin modifier pathway, such as *KMT2C* (14%), *KMT2D* (14%), *BAP1* (9%), *ARID1A* (5%), and others, were highly mutated in NPC (Supplementary Fig. S2A). There was inferior PFS for patients (13 of 22; 59.1%) with mutations in chromatin modifier gene [median PFS 8.6 months (95% CI, 0–17.7 months)] compared with patients with wild-type of chromatin modifier gene [median PFS 18.5 months (95% CI, 13.4–23.5 months); Supplementary Fig. S2B]. The median OS showed an inferior tendency in patients with mutations in chromatin histone modifiers (Supplementary Fig. S2C).

SCNA levels can predict therapy response

We analyzed SCNA in NPC depending on the chromosome arm levels (broad region; **Fig. 3A**). To further understand the poor response in patients with high-SCNA levels, we used ESTIMATE algorithm to investigate tumor microenvironment signatures and performed GSEA. Patients with high SCNA levels (>3 points) showed lower immune scores (ESTIMATE) than those with low SCNA levels (≤3 points; 658 vs. 1,183; Wilcox $P < 0.01$; **Fig. 3B**). High SCNA levels were associated with a poor response to nivolumab plus gemcitabine. Patients with CR or PR showed relatively low SCNA levels (median = 2 points; range 0–4) compared with patients with SD or PD (median = 5.5 points; range 2–23; Wilcox $P < 0.01$; **Fig. 3C**). Median PFS were 3.6 months (95% CI, 1.2–6.0) in patients with high SCNA levels ($n = 9$) and 16.8 months (95% CI, 12.9–20.6) in patients with low SCNA levels ($n = 13$; $P < 0.001$; **Fig. 3D**). The median OS was not reached in patients with a low SCNA level and

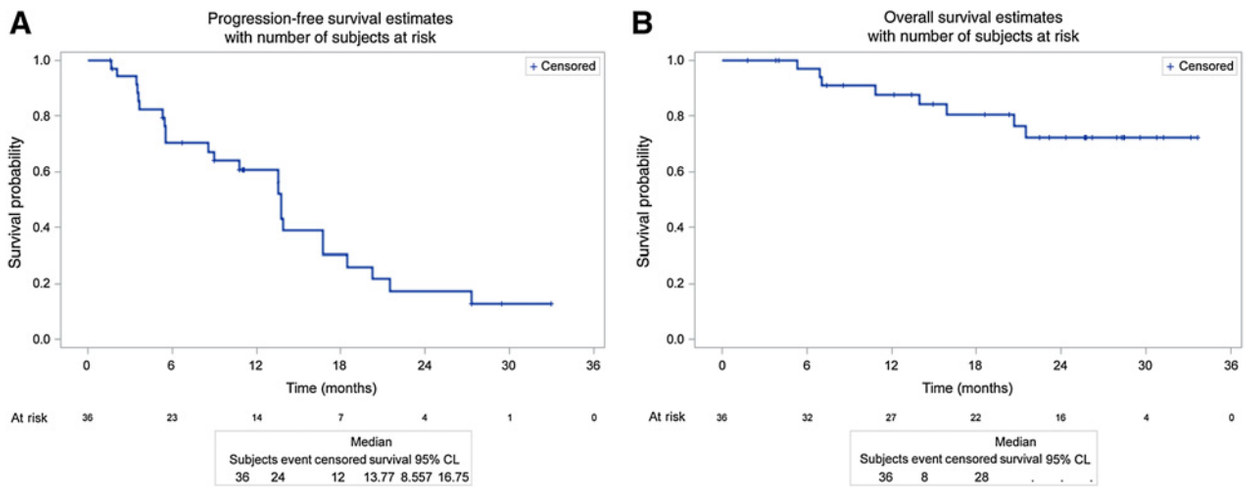

Figure 2.
Kaplan–Meier curves for (**A**) PFS and (**B**) OS.

Table 3. Treatment-related adverse event of nivolumab plus gemcitabine.

Adverse event	Number of patients (%)			
	Total	Grade 1	Grade 2	Grade 3
Nausea	8 (22.2)	5 (13.9)	3 (8.3)	0
Anorexia	6 (16.7)	2 (5.6)	4 (11.1)	0
Acneiform rash	6 (16.7)	3 (8.3)	3 (8.3)	0
Fatigue	5 (13.9)	2 (5.6)	3 (8.3)	0
Fever	4 (11.1)	2 (5.6)	2 (5.6)	0
Pruritus	3 (8.3)	1 (2.8)	2 (5.6)	0
Maculo-papular rash	3 (8.3)	2 (5.6)	1 (2.8)	0
Generalized muscle weakness	3 (8.3)	2 (5.6)	1 (2.8)	0
Vomiting	2 (5.6)	0	2 (5.6)	0
Diarrhea	2 (5.6)	0	2 (5.6)	0
Hypothyroidism	2 (5.6)	0	2 (5.6)	0
Hypertension	2 (5.6)	0	1 (2.8)	1 (2.8)
Anemia	2 (5.6)	0	1 (2.8)	1 (2.8)
Oral mucositis	2 (5.6)	1 (2.8)	1 (2.8)	0
Arthralgia	2 (5.6)	1 (2.8)	1 (2.8)	0
Alanine aminotransferase increased	2 (5.6)	2 (5.6)	0	0
Cough	1 (2.8)	0	1 (2.8)	0
Dry mouth	1 (2.8)	0	1 (2.8)	0
Epistaxis	1 (2.8)	0	1 (2.8)	0
Fracture	1 (2.8)	0	1 (2.8)	0
Glaucoma	1 (2.8)	0	1 (2.8)	0
Localized edema	1 (2.8)	0	1 (2.8)	0
Mucosal infection	1 (2.8)	0	1 (2.8)	0
Pneumonitis	1 (2.8)	0	1 (2.8)	0
Dyspnea	1 (2.8)	0	1 (2.8)	0
INR increased	1 (2.8)	0	1 (2.8)	0
Fatigue	1 (2.8)	0	1 (2.8)	0
Weight loss	1 (2.8)	0	1 (2.8)	0
Allergic reaction	1 (2.8)	1 (2.8)	0	0
Gastritis	1 (2.8)	1 (2.8)	0	0
Headache	1 (2.8)	1 (2.8)	0	0
Urinary incontinence	1 (2.8)	1 (2.8)	0	0
Papulopustular rash	1 (2.8)	1 (2.8)	0	0
Urticaria	1 (2.8)	1 (2.8)	0	0
Alopecia	1 (2.8)	1 (2.8)	0	0
Aspartate aminotransferase increased	1 (2.8)	1 (2.8)	0	0

a high SCNA level; however, the median OS was inferior in patients with high SCNA levels compared with patients with low SCNA levels ($P = 0.0077$; **Fig. 3E**).

Figure 3F shows the pathways in Gene Ontologies and Hallmark were enriched in the differential gene expression data in the high-SCNA group versus the low-SCNA group. Immune-related pathways, such as inflammatory response, activation of immune response, and humoral immune response, were downregulated in the high-SCNA group, whereas cell proliferation–related pathways were enriched in the high-SCNA group (Supplementary Fig. S3; Supplementary Table S2).

Median tumor mutational burden (TMB) was 2.1 mut/Mb (range 0.3–6.3) and TMB had a positive correlation with SCNA levels ($P = 0.03$; Supplementary Fig. S4). However, high TMB (≥2.1) was not related to either ORR ($P = 1.0$) or PFS ($P = 0.37$).

In multivariate analysis of mutation status of chromatin modifier gene (mutation vs. wild-type), TMB (high TMB vs. low TMB), and SCNA level (high SCNA vs. low SCNA), high SCNA had significantly inferior PFS compared with patients with low SCNA in multivariate analysis, (HR, 7.0; 95% CI, 1.3–37.9; $P = 0.02$).

Immune-excluded immune phenotype was correlated with poor clinical outcomes

We performed immune phenotype analysis based on Lunit SCOPE IO, an artificial intelligence–powered spatial TIL analyzer from H&E images of corresponding tumor samples ($n = 24$; **Fig. 4A**; Supplementary Tables S3 and S4). Interestingly, two thirds ($n = 16$ of 24) of samples harbored inflamed immune phenotype predominantly (>50% of WSI), whereas the proportion of immune-excluded immune phenotype [immune-excluded score (IES)] was widely distributed according to the samples (median 9.0%; range 0%–44.8%; **Fig. 4B**). Patients with CR or PR showed significantly lower IES than those with SD or PD (Wilcox $P < 0.05$; **Fig. 4C**). Median PFS was 18.5 months (95% CI, 13.8–not reached) in patients with low IES (≤9.0%; $n = 12$) and 10.8 months (95% CI, 5.4–not reached) in patients with high IES (>9.0%; $n = 12$; $P < 0.05$; **Fig. 4D**). Median OS was not reached in both patients with low IES (≤9.0%) and high IES (>9.0%); however, median OS was inferior in patients with high IES compared with patients with low IES (≤9.0%; $P = 0.162$; **Fig. 4E**).

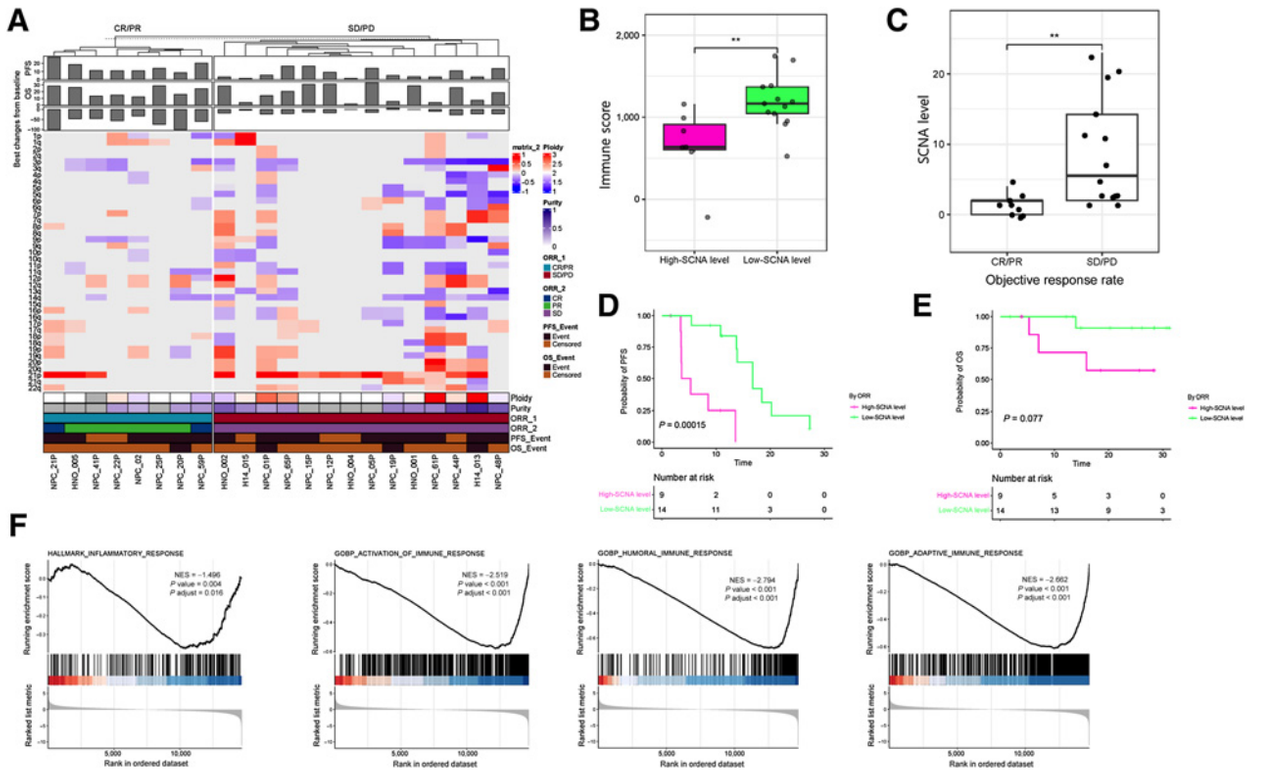

Figure 3.

A, The levels of broad SCNA. **B,** Immune score (estimation of stromal and immune cells in malignant tumor tissues using expression data) by SCNA level. **C,** SCNA level by objective response. **D,** PFS. **E,** OS by SCNA level (high SCNA >3, low SCNA ≤ 3). **F,** Immune-related pathways in Hallmark or Gene Ontology.

Discussion

This study showed that nivolumab plus gemcitabine had promising efficacy and a favorable safety profile in patients with recurrent or metastatic NPC who progressed to platinum-based chemotherapy.

A recent study showed that anti–PD-1/PD-L1 monotherapy increased survival with moderate antitumor activity in patients with recurrent or metastatic NPC who progressed to platinum-based chemotherapy. Nivolumab showed the ORR of 20.5% and 1-year PFS rate of 19.3% (7). In KEYNOTE-028 study, pembrolizumab showed that ORR was 25.9%, median PFS was 6.5 months, and median OS was 16.5 months. Toripalimab also showed the ORR of 23.9% with median PFS of 1.9 months and median OS of 15.1 months in patients with previously treated recurrent or metastatic NPC (16). In phase III, pembrolizumab monotherapy did not improve OS compared with chemotherapy (17).

Compared with a previous study of anti–PD-1/PD-L1 monotherapy, nivolumab plus gemcitabine showed superior PFS and OS in this study. Gemcitabine enhances antitumor immune activity (9). One potential reason for the promising outcomes of nivolumab plus gemcitabine may be the synergistic effect. The role of the T-cell attractant gemcitabine led to a synergistic effect to improve the response and survival outcomes of nivolumab plus gemcitabine in patients with NPC. In JUPITOR-02 trial, gemcitabine plus cisplatin was administered for up to six cycles (18). As this study showed manageable toxicity, potential benefit of continuation of gemcitabine and nivolumab might be expected as a maintenance therapy after 6 cycles in the further first-line study.

The adverse event of nivolumab plus gemcitabine were as expected; drug-induced adverse events of nausea and mucositis were more frequent with gemcitabine, and rash was associated with nivolumab. However, all treatment-related adverse events were tolerable and manageable. QOL assessment showed that the global health status/QOL, functional scales, and symptom scales tended to improve from baseline to week 18. However, from baseline to week 24, these scales did not show significant changes, and were maintained at baseline.

In a previous study, as potential genomic biomarkers of PD-1/PD-L1 inhibitor in patients with recurrent or metastatic NPC, chromatin histone modifiers such as *ARID1A* (10%), *MLL2* (6%), and *BAP1* (4%) were the most frequent genomic alterations (19). In the study of WES and whole-genome sequencing on micro-dissected NPC, they revealed a higher digress of somatic mutation and several novel genomic events such as multiple negative regulators of the NF-kB pathway including CYLD, TRAF3, NFKB1A, and NLRC5 (20). In our study, approximately half of the patients had mutations in the chromatin histone modifier and had inferior PFS compared with the wild-type.

Aneuploidy, also known as SCNA, is associated with tumorigenesis. High SCNA in a broad region was associated with immune evasion and showed poor response to immunotherapy (21). SCNA was a stronger predictor of cytotoxic immune cell infiltration than tumor mutational load. In our study, there was a positive correlation between TMB and SCNA levels, as in a previous study; however, TMB did not predict the ORR and PFS of nivolumab plus gemcitabine treatment in patients

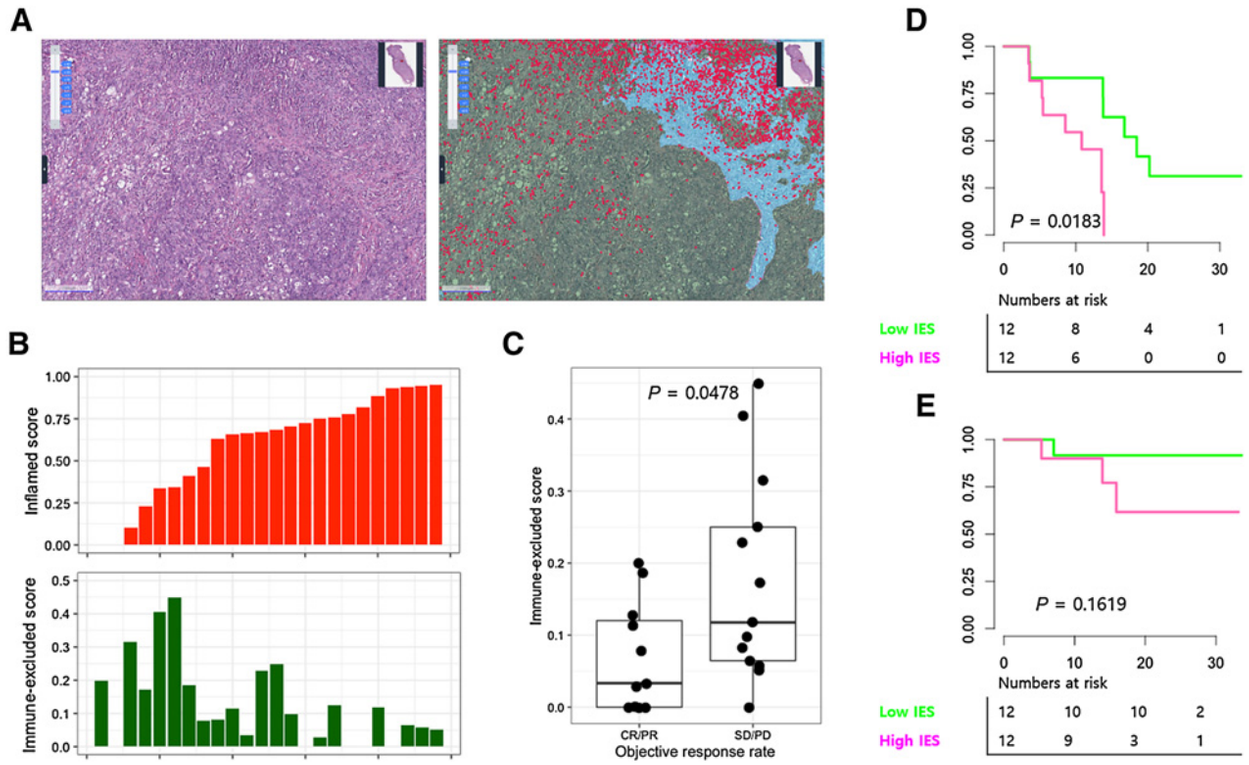

Figure 4.

Immune phenotype analysis. **A,** Representative image of H&E original image (left) and Lunit SCOPE IO inferenced segmentation of cancer epithelium (green), cancer stroma (blue), and TIL (red), respectively (right). **B,** Proportion of inflamed area (inflamed score, red; top) and immune-excluded area (immune-excluded score, green; bottom). **C,** Immune-excluded score by ORR. **D,** PFS by immune-excluded score. **E,** OS by immune-excluded score.

with recurrent or metastatic NPC. In a study of toripalimab, TMB had no predictive value for response in NPC (16). Interestingly, immune phenotype based on spatial analysis of TIL in pathology images showed that the proportion of immune-excluded immune phenotype, or dense stromal TIL area, was correlated with inferior ORR and PFS of gemcitabine and nivolumab combination, indicating that TIL exclusion outside of the cancer area is one of the resistance mechanisms of the combination regimen in NPC. The objective of identifying biomarkers is an exploratory analysis in this study. The artificial intelligence–powered spatial TIL analyzer description would be needed to be further validated.

Notably, patients with high SCNA (>3 points) had inferior ORR and PFS compared with those with low SCNA (≤3 points). Through multivariate analysis, the high SCNA only predicted lower ORR and inferior PFS in patients receiving nivolumab plus gemcitabine treatment significantly (HR, 7.0; 95% CI, 1.3–37.9; $P = 0.02$). Consistently, the level of SCNA reflected antitumor immunity; pathways such as inflammatory response, activation of immune response, and humoral immune response were downregulated in patients with high SCNA levels. However, the impact of these genomic alterations as biomarkers for anti–PD-1/PD-L1 inhibitors plus chemotherapy in patients with NPC needs to be further investigated.

Conclusion

Nivolumab plus gemcitabine had promising clinical activity for patients with recurrent or metastatic NPC, demonstrating a high ORR, prolonged PFS, and OS with manageable toxicity. Immune-excluded immune phenotype was associated with poor PFS and needs to be further validated.

Authors' Disclosures

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Disclaimer

This study was an investigator-sponsored trial, and the study was conducted independently of the funder. The funder had no role in the study design, data collection, data analysis, or data interpretation. The investigators collected, analyzed, and interpreted the data. All authors had full access to the data. The corresponding

author had full access to all data and had final responsibility for the decision to submit for publication.

Authors' Contributions

H.A. Jung: Conceptualization, resources, data curation, software, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **K.-U. Park:** Resources, validation, investigation, writing–review and editing. **S. Cho:** Resources, data curation, investigation, writing–original draft, writing–review and editing. **J. Lim:** Data curation, formal analysis, writing–original draft, writing–review and editing. **K.-W. Lee:** Data curation, supervision, writing–original draft, writing–review and editing. **M.H. Hong:** Supervision, validation, investigation, writing–review and editing. **T. Yun:** Validation, investigation, writing–original draft, writing–review and editing. **H.J. An:** Validation, investigation, writing–review and editing. **W.-Y. Park:** Software, validation, writing–review and editing. **S. Pereira:** Data curation, formal analysis, visualization, writing–review and editing. **C.-Y. Ock:** Formal analysis, supervision, validation, investigation, writing–original draft, writing–review and editing. **B. Keam:** Conceptualization, resources, data curation, software, formal analysis, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing.

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Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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