GX-188E DNA vaccine plus pembrolizumab in HPV 16- and/or 18-positive recurrent or advance cervical cancer: a phase 2 trial

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Summary

Background In an interim analysis of this phase 2 trial, adding the GX-188E vaccine to pembrolizumab resulted in manageable toxicity with antitumor activities in patients with recurrent or advanced cervical cancer. Here, we report the final safety and efficacy results after a long-term follow-up at the study's completion.

Methods This open-label, single-arm, phase II trial was conducted in nine hospitals in South Korea (ClinicalTrials.gov identifier, NCT03444376). Eligible patients were aged ≥18 years with recurrent or advanced inoperable cervical cancer, Eastern Cooperative Oncology Group Performance status of 0 or 1, and positivity for HPV 16/18, who failed the available standard-of-care therapy. Patients received intramuscular 2 mg GX-188E at weeks 1, 2, 4, 7, 13, 19, and 46 and intravenous 200 mg pembrolizumab every 3 weeks for up to 2 years or until disease progression. The primary endpoint was the objective response rate (ORR) within 24 weeks.

Findings Between June 19, 2018, and December 24, 2021, 65 patients were enrolled and received at least one dose of the study treatment. Sixty patients received combination treatment with GX-188E and pembrolizumab and underwent efficacy analysis. After a median follow-up of 14.72 months, the confirmed ORR was 35.0% (95% CI, 23.1–48.4). Five patients (8.3%) had a complete response, and 16 (26.7%) had a partial response. In addition, patients with PD-L1-positive and PD-L1-negative tumors had an ORR of 38.9% (95% CI, 23.1–56.5) and 29.2% (95% CI, 12.6–51.1), respectively. The median duration of response of all the patients was 12.3 months (95% CI, 5.3–not reached [NR]). For those with PD-L1-positive tumors, it was 12.3 months (95% CI, 3.5–NR), and for those with PD-L1-negative tumors, it was NR (95% CI, 2.4–NR). The median progression-free survival of the 60 patients was 4.4 months (95% CI, 2.1–8.3), and the median overall survival was 23.8 months (95% CI, 14.0–NR). 22 (33.8%) of 65 patients had treatment-related adverse events (TRAEs) of any grade and four (6.2%) had grade 3–4 TRAEs. No treatment-related deaths occurred.

Interpretation The GX-188E vaccine combined with pembrolizumab in recurrent or advanced HPV-positive cervical cancer was safe and showed a promising overall survival and clinical response rate. This combination therapy might provide a new potential treatment option for patients with recurrent or advanced cervical cancer.

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Keywords: Cervical cancer; DNA vaccine; HPV; Pembrolizumab; Overall survival

Research in context

Evidence before this study

We searched PubMed through November 30, 2023, for recurrent or advanced cervical cancer trials using the terms "HPV-positive", "checkpoint inhibitor", "PD-L1", "ORR, "OS", and "clinical trial" without language restriction.
Most clinical trials using checkpoint inhibitors alone or in combination showed favorable ORR in PD-L1-positive patients, and only a few trials reported a limited ORR in PD-L1-negative patients. Therefore, it is crucial to explore alternative therapeutic options for recurrent or advanced cervical cancer regardless of PD-L1 expression.

Added value of this study

This study demonstrates that the combination of the GX-188E vaccine with pembrolizumab for treating recurrent or advanced HPV-positive cervical cancer is a safe treatment modality that may offer promising overall survival rates and clinical response rates regardless of PD-L1 expression.

Implications of all the available evidence

If further studies confirm these findings, this combination therapy might provide a new potential treatment option for patients with recurrent or advanced cervical cancer.

Introduction

Cervical cancer is the fourth most frequently diagnosed and fourth leading cause of cancer-related death in women.[1] Persistent Human papillomavirus (HPV) is associated with >95% of all cervical cancer cases, and HPV-16 and HPV-18 genotypes are associated with 71% of cervical cancer cases.[2–4]

For patients with recurrent or advanced cervical cancer, the use of platinum-containing doublet combinations, either with or without bevacizumab, has been established as the current standard first-line systemic therapy.[5–7] Furthermore, recent research has demonstrated that immunotherapy, such as pembrolizumab and atezolizumab, when combined with platinum-based chemotherapy, with or without bevacizumab, as first-line therapy offers clinically significant improvements in both PD-L1-positive and -negative patients with recurrent or metastatic cervical cancer.[8,9] However, there is still a high unmet need regarding this disease, given its dismal prognosis and the scarcity of effective treatment options after the failure of first-line systemic therapies.

Pembrolizumab has been approved by the US Food and Drug Administration (FDA) for patients with recurrent or advanced cervical cancer with disease progression after one or more previous chemotherapies for programmed death ligand 1 (PD-L1)-positive tumors. The approval was granted based on the KEYNOTE-158 trial results, which demonstrated a higher objective response rate (ORR) of 14.3% in PD-L1-positive patients compared to other systemic therapies, which have shown an ORR of less than 15%.[10] Cemiplimab has been approved by the European Medicines Agency (EMA) for the treatment of adult patients with recurrent or metastatic cervical cancer and disease progression on or after platinum-based chemotherapy based on EMPOWER-cervical 1 trial results, which demonstrated improved overall survival (OS) in the intention to treat population, and also in PD-L1 negative tumors.[11]

It has been indicated that the expression of PD-L1 in cervical cancer ranges from 16.7% to 34.4%.[12] Given that pembrolizumab monotherapy is only approved for patients whose tumors express PD-L1, and that cemiplimab monotherapy also has a limited ORR of approximately 10% in PD-L1-negative patients, it is crucial to explore alternative therapeutic options for cervical cancer in patients with negative PD-L1 expression.

GX-188E (tirvalimogene teraplasmid), a therapeutic DNA vaccine that encodes HPV-16 and HPV-18 E6 and E7 proteins, is designed to enhance the processing and presentation of HPV E6/E7 antigens by dendritic cells through the co-expression of Fms-like tyrosine kinase-3 ligand.[13] Previous studies on various HPV therapeutic vaccines alone or with other therapies (conventional chemotherapies or immunotherapies) observed T-cell-induced immunity, yielding antitumor activity in HPV-infected tumor cases.[14] In previously reported interim results of this phase 2 trial, the administration of GX-188E and pembrolizumab yielded promising anti-tumor activities, with a confirmed ORR of 42%. The treatment was generally safe and well-tolerated, with manageable adverse events.[15] Herein, we present the final efficacy and safety results, including the primary endpoint (ORR) of this combination therapy in the treatment of recurrent or advanced cervical cancer that has been previously treated.

Methods

Study design and participants

This was a multicenter, open-label, single-arm phase II study (ClinicalTrials.gov identifier, NCT03444376). Patients were recruited from nine hospitals in Korea: the National Cancer Center, Asan Medical Center, Catholic University of Seoul St. Mary's Hospital, Seoul National University Bundang Hospital, Samsung Medical

Center, Keimyung University Dongsan Medical Center, Yonsei University College of Medicine, Inje University Busan Paik Hospital, and Korea University Guro Hospital.

Eligible patients were women aged 18 years or older and had histologically confirmed recurrent or advanced cervical cancer with HPV infection (HPV-16 and/or –18); had progressed on or were intolerant to first or later lines of chemotherapy; had measurable disease based on the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1), as evaluated by the blinded independent central review (BICR); and had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Patients with a history of active central nervous system metastases or active autoimmune disease, an allogeneic solid organ or bone marrow transplant, or a diagnosis of immunodeficiency were excluded.

Ethics

The trial protocol and other materials were approved by the institutional review boards or ethics committees of the nine hospitals described above subsection, and the Ministry of Food and Drug Safety (MFDS), the competent authority in Korea. Written informed consent was obtained from all participants. The full protocol is available in Supplementary Material. The trial was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines and the principles of the Declaration of Helsinki.

Procedures

GX-188E (2 mg) was administered intramuscularly either into deltoid or lateralis muscles using electroporation (TriGrid Delivery System, Ichor Medical Systems, Inc.) at weeks 1, 2, 4, 7, 13, and 19 with one optional dose at week 46. The vaccination schedule was consistent with the administration schedule of pembrolizumab, except for an additional dose administered at week 2 to induce a T-cell response as soon as possible. Pembrolizumab (200 mg) was administered using an intravenous infusion on day 1 of each 3-week treatment cycle. In case of grade 3 or higher hematological or non-hematological adverse events defined as dose-limiting toxicity, the patients were advised to skip the GX-188E treatment at week 2 and have their pembrolizumab treatment interrupted or reduced. The duration of treatment was approximately 2 years or until disease progression.

Tumor PD-L1 expression was analyzed at a central laboratory using the IHC 22C3 pharmDx assay (Agilent Technologies, Carpinteria, CA) and was considered positive when the combined positive score (CPS) was ≥1. Peripheral blood samples were taken from the patients at screening and weeks 1, 4, 7, 10, 16, 22, and 46 for IFN-r ELISpot assays (BD Biosciences, Franklin Lakes, USA). To study the cellular immune response to GX-188E, we analyzed HPV-16 and HPV-18 E6-specific and E7-specific T-cell responses. T-cell responses to HPV E6 and E7 were determined by comparing the signals with baseline levels. Reports indicate that a 3-fold increase in IFN-γ ELISpot signals over baseline is considered positive.

Outcomes

The primary endpoint was the ORR within 24 weeks based on the BICR at screening according to RECIST version 1.1. Confirmatory scans were acquired for all determinations of objective response (partial response [PR] or complete response [CR]), stable disease, and disease progression (PD). To evaluate tumor response, radiographical evaluations of tumor burden were performed every 9 weeks during the treatment period or more frequently if clinically indicated and at the end of the study if disease progression was confirmed radiographically. The secondary endpoints included, but were not limited to, the best overall response rate (BORR) based on RECIST v1.1 and RECIST for Immunotherapeutics, time-to-best response (TTR), duration of response (DOR), progression-free survival (PFS; 6-month PFS rate and median PFS time), overall survival (OS; 6-month OS rate and median OS time), and disease control rate (DCR).

An efficacy analysis was also performed in the safety population as a sensitivity analysis. The ORR and DCR were also assessed in pre-specified subgroups (Age, HPV type, histology type, prior bevacizumab, ECOG performance status, and PD-L1 status) using a statistical analysis plan. Analysis of the ORR based on prior radiotherapy, previous surgical treatment, and previous lines of the systemic regimen was conducted post hoc.

The safety and tolerability of the study treatment regimen was also evaluated. Safety analyses included all participants who received at least one dose of the study treatment. The adverse events (AEs) experienced by patients were comprehensively evaluated. The investigators evaluated the severity of the AEs based on the Common Terminology Criteria for Adverse Events (CTCAE v4.03) by the National Cancer Institute.

Statistics

The data cutoff was June 9, 2023. Sixty-five patients were enrolled and received at least one dose of the study treatment for this phase Ib/II trial, defined as the safety population. Sixty patients were defined as the efficacy evaluable population based on having at least one post-baseline tumor assessment with at least 45 days of study treatment. The primary and secondary efficacy endpoints were analyzed in safety and efficacy evaluable populations, respectively. The study size of 60 participants in Part C was determined using the ORR in the total population reported in the Keynote-158 study (pembrolizumab monotherapy in patients who had progressed with the standard-of-care systemic therapy)

as a historical control. With a null hypothesis for the ORR set at P0 = 12.2% and an alternative hypothesis for ORR of Pa = 37%, the sample size of 60 participants was calculated considering a two-sided significance level of 0.05, 97% power, and a 25% dropout rate. The primary endpoint of the ORR was estimated as the binomial proportion of the best overall response of a confirmed PR or CR within the first 24 weeks of treatment and reported using a two-sided, 95% Wilson score confidence interval (CI). Kaplan–Meier methods were used to estimate medians and 95% CIs for the PFS and OS. Descriptive statistics were used to summarize trial results, and categorical variables were summarized using counts and percentages. The Cox regression analyses for both PFS and OS were conducted post-hoc. P-values were based on log-rank test for PD-L1-positive and PD-L1-negative groups.

AEs were coded according to the MedDRA adverse event dictionary. The results were tabulated to examine their frequency, the organ systems they affected, and their relationship to the study treatment. Efficacy results, including ORR, were analyzed using descriptive statistics.

Role of the funding source

The sponsor of the study had a role in study design, data collection, data analysis, data interpretation, and writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Patients

Between June 19, 2018, and December 24, 2021, 65 female patients were enrolled and received at least one dose of the study treatment. Sixty patients underwent at least 45 days of treatment with GX-188E and pembrolizumab and efficacy analysis (Fig. 1). The baseline demographic and disease characteristics of the study population are summarized in Table 1. The median age was 52 years (range, 27–79 years). Thirty-nine patients (60.0%) had PD-L1-positive tumors (CPS ≥1), and 26 (40.0%) had PD-L1-negative tumors. The histologic types were squamous cell carcinoma (SCC) (n = 50, 76.9%) and adenocarcinoma (AC) (n = 15, 23.1%). Thirty-two SCCs (64% of all SCCs) and eight ACs (53.3% of all ACs) were PD-L1-positive. Forty-nine patients had HPV-16, and 16 had HPV-18 or both.

At the time of data cutoff, two (3.1%) patients were still undergoing the study treatment, and the median follow-up period for all 65 patients was 14.72 months.

Efficacy

The confirmed ORR of the 60 patients based on the efficacy analysis within 24 weeks were 35.0% (95% CI, 23.1–48.4) and included five CRs (8.3%) and 16 PRs (26.7%) (Table 2). The BORR of the 60 patients (efficacy population) was also 35.0% (95% CI, 23.1–48.4) and included seven CRs (11.7%) and 14 PRs (23.3%). Although there was a difference in the number of CRs because two responders changed from PR to CR after 24 weeks, there was no difference in the response rate within 24 weeks or the best ORR for the entire period. Therefore, the term ORR was unified and indicated as ORR.

For sensitivity analysis, the ORR of the 65 patients (safety population) was 32.3% (95% CI, 21.2–45.1). The tumor assessment of five patients not included in the efficacy evaluable population included one SD, one PD, and three 'Not Evaluables' because three patients did not undergo post-baseline tumor assessment.

In addition, patients with PD-L1 (+) and (–) tumors had ORRs of 38.9% (95% CI, 23.1–56.5%) and 29.2% (95% CI, 12.6–51.1%), respectively. The durability and timing of the objective responses are presented according to PD-L1-positive and PD-L1-negative tumors and illustrated in Fig. 2A. All 17 of the 21 patients exhibited an objective response within 3 months, and the median TTR was 2.1 months (95% CI, 2.07–3.02). Six out of seven patients with CR continued to have a complete response until the end of the study, and the remaining one with CR had new lesions with stable disease at 9 months. The median DOR of all the patients was 12.3 months (95% CI, 5.3–not reached [NR]). For those with PD-L1-positive tumors, it was 12.3 months (95% CI, 3.5–NR), and for those with PD-L1-negative tumors, it was NR (95% CI, 2.4–NR).

The best change from baseline in target lesion size and changes in tumor burden over time for all the patients are illustrated in Fig. 2B. The best changes from the baseline did not show any statistical differences among the CR, PR, SD, and PD groups when subgrouped into PD-L1-positive and PD-L1-negative tumor groups (Appendix 1). Among the responders, the ongoing confirmed DOR ≥6 months and ≥12 months were 61.9% (95% CI, 38.4–81.9) and 42.9% (95% CI, 21.8–66.0), respectively. Additionally, we analyzed the overall response rate according to the pre-specified subgroups, and the results did not show any statistical differences according to the number of variables (Appendix 2), including age, histology, PD-L1 status, HPV types, number of previous systemic chemotherapies, history of bevacizumab use, ECOG status, history of treatment-related surgery, and surgery types (minimally invasive or open surgery).

The median PFS of the 60 patients was 4.4 months (95% CI, 2.1–8.3), and the Kaplan–Meier estimate of the probability of PFS ≥6 months was 45.0% (95% CI, 32.1–58.4) (Table 2 and Fig. 3). The median OS was 23.8 months (95% CI, 14.0–NR), and the Kaplan–Meier estimates of the probability of OS ≥ 6 and ≥ 12 months were 90.0% (95% CI, 79.5–96.2) and 58.3% (95% CI, 44.9–70.9), respectively. The effect of the combination therapy on the median PFS and OS according to the PD-L1 expression status is illustrated in Fig. 3. The

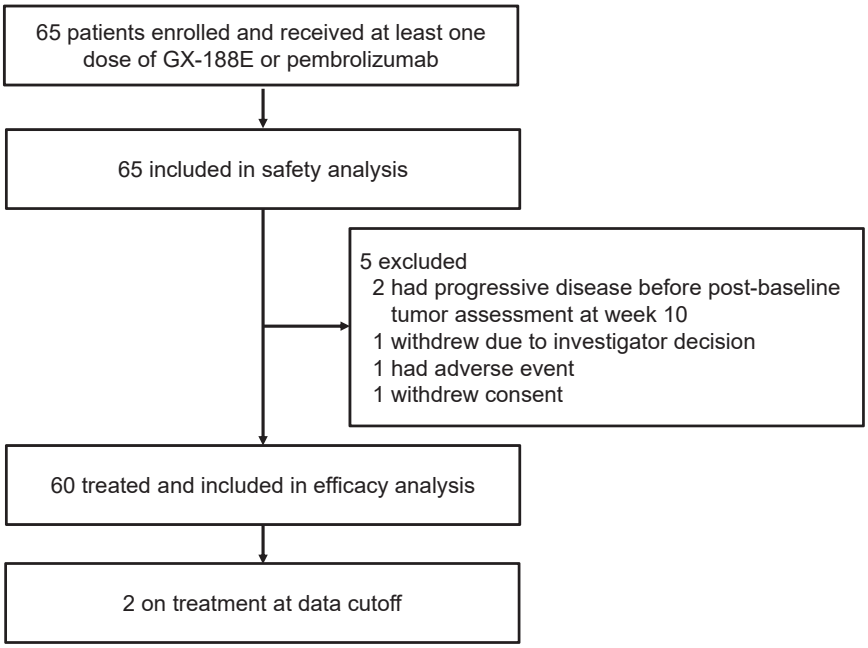

Fig. 1: **Trial profile and patient disposition.**

Characteristic	Patients (N = 65)
Age, years	
Median (range)	52 (27–79)
PD-L1 expression status	
Positive (CPS ≥ 1)	39 (60.0)
Negative (CPS < 1)	26 (40.0)
Histologic type	
Squamous cell carcinoma	50 (76.9)
Adenocarcinoma	15 (23.1)
HPV type	
HPV-16	49 (75.4)
HPV-18 or both[a]	16 (24.6)
Number of previous systemic therapies	
0	2 (3.1)
1	31 (47.7)
2	21 (32.3)
3	11 (16.9)
Previous bevacizumab use	
Yes	45 (69.2)
No	20 (30.8)
Previous surgery	
Yes	38 (58.5)
No	27 (41.5)
ECOG performance status score	
0	34 (52.3)
1	31 (47.7)

Abbreviations: CPS, Combined positive score; ECOG, Eastern Cooperative Oncology Group performance; PD-L1, Programmed death ligand-1. Note: Data are presented as No. (%) unless indicated otherwise. [a]Both HPV-16 and -18.

Table 1: **Baseline demographic and disease characteristics.**

patients with CPSs ≥1 and < 1 exhibited median PFSs of 5.1 months and 2.2 months, respectively (Hazard ratio [HR], 0.72; 95% CI, 0.40–1.31). The median OS of the patients with CPSs ≥1 and < 1 were 28.2 months and 14.0 months, respectively (HR, 0.56; 95%, CI, 0.27–1.18). No statistically significant findings were found in the univariate Cox regression model for PFS and OS with PD-L1 expression (P > 0.1). Additionally, no statistically significant findings were observed in the multivariate Cox regression model with PD-L1 expression and HPV type (P > 0.1).

In the pre-specified exploratory analysis, HPV 16- or/and 18-E6/E7-specific T-cell responses induced by GX-188E, IFN-γ ELISpot was analyzed. Exactly 100 PBMC samples from 50 patients at screening and 4 weeks after the first injection were available. The paired sample t-test analysis revealed the increase in HPV 16- and HPV 18-E6/E7-specific T-cell responses after 4 weeks from the first GX-188E administration among responders with statistical significance (P = 0.036), but not among non-responders (P = 0.355). Statistical differences were observed among responders and non-responders between antigen-specific responses at 4 weeks after the first injection and at screening (IFN-γ ELISpot analysis at 4 weeks after the first injection and at screening) (Appendix 3).

Safety

Twenty-two patients (33.8%) had TRAEs of any grade. The most common TRAEs of any grade were hypothyroidism (13.8%), diarrhea (3.1%), nausea (3.1%), vomiting (3.1%), urticaria (3.1%), and increased blood

Response of the patients included in efficacy analysis	Patients (n = 60)	Patients (n = 65)
Objective response rate within 24 weeks (%, 95% CI)	21 (35.0, 23.1–48.4)	21 (32.3, 21.2–45.1)
Best overall response within 24 weeks		
Complete response (CR)	5 (8.3)	5 (7.7)
Partial response (PR)	16 (26.7)	16 (24.6)
Stable disease (SD)	13 (21.7)	14 (21.5)
Progression of disease (PD)	26 (43.3)	27 (41.5)
Not Evaluable (NE)	0	3 (4.6)
Best objective response rate (%, 95% CI)	21 (35.0, 23.1–48.4)	21 (32.3, 21.2–45.1)
Best overall response for the entire period		
Complete response (CR)	7 (11.7)	7 (10.8)
Partial response (PR)	14 (23.3)	14 (21.5)
Stable disease (SD)	13 (21.7)	14 (21.5)
Progression of disease (PD)	26 (43.3)	27 (41.5)
Not Evaluable (NE)	0	3 (4.6)
Disease control rate[a]	34 (56.7, 43.2–69.4)	35 (53.8, 41.0–66.3)
Median (95% CI) duration of response (months)[b]	21 (12.3, 5.3-NR)	21 (12.3, 5.3-NR)
Ongoing confirmed response ≥6 months (95% CI)	61.9% (38.4–81.9)	61.9% (38.4–81.9)
Ongoing confirmed response ≥12 months (95% CI)	42.9% (21.8–66.0)	42.9% (21.8–66.0)
Median (95% CI) time-to-best response (months)[c]	21 (2.1, 2.07–3.02)	21 (2.1, 2.07–3.02)
Median (95% CI) progression-free survival (months)	4.4 (2.1–8.3)	4.3 (2.1–8.3)
6-months progression-free survival rate (95% CI)	45.0% (32.1–58.4)	42.9% (30.5–56.0)
Median (95% CI) overall survival (months)	23.8 (14.0-NR)	17.2 (12.0-NR)
6-months overall survival rate (95% CI)	90.0% (79.5–96.2)	86.2% (75.3–93.5)
12-months overall survival rate (95% CI)	58.3% (44.9–70.9)	55.4% (42.5–67.7)
24-months overall survival rate (95% CI)	23.3% (13.4–36.0)	21.5% (12.3–33.5)

Note: Data are presented as No. (%) unless indicated otherwise. Abbreviations: CI, Confidence interval; NR, Not reached. [a]Defined as proportion of patients with a confirmed CR, PR, or SD without progression for at least 24 weeks. [b]Evaluated in patients who had a CR or PR (n = 21). [c]Time from treatment initiation to the best objective response achieved. This endpoint is only determined for subjects who have a PR or CR.

Table 2: **Tumor response assessment by blinded independent central review (BICR).**

thyroid stimulating hormone levels (3.1%) (Table 3). The overall incidence of ≥ grade 3 TRAEs was 6.2% with increased aspartate aminotransferase (AST) levels, increased alanine aminotransferase (ALT) levels, decreased neutrophil count, and syncope. Syncope occurred after the administration of GX-188E (on week 4 day 1) in one participant and resolved within 1 min. This adverse event was not considered dose-limiting toxicity (DLT) because it did not occur within the DLT-evaluation period based on the DLT definition in the protocol (Supplementary Material). Moreover, one patient had a TRAE that led to study discontinuation due to increased AST (grade 3) and ALT (grade 4) levels but recovered to normal levels after drug administration was discontinued. Therefore, no TRAE-related deaths occurred. Immune-related AEs (irAEs) were observed in 14 patients (21.5%) (Appendix 4). No irAE-related deaths occurred, and other irAEs were stomatitis (1.5%), pruritis (1.5%), and pyrexia (1.5%).

Discussion

In this phase 2 trial of the GX-188E DNA vaccine plus pembrolizumab for HPV 16- and/or 18- positive, PD-L1 all-comer pretreated recurrent and advanced cervical cancer, we observed clinical activity in patients treated after the failure of at least one line of systemic therapy. The ORR was 35.0%, including 8.3% of the patients who experienced a CR following the combination therapy. We found that targeting HPV through combination therapy with pembrolizumab led to clinical improvements in the size of target lesions among patients with recurrent, advanced cervical cancer. In addition, we found that clinically meaningful activity resulted from increasing the median OS (23.8 months) irrespective of several variables, including PD-L1 expression and HPV type.

Currently, there is no established subsequent therapy after the failure of frontline treatment, as previous research on the monotherapy of several anti-cancer treatments has demonstrated low response rates.[16,17] Even though this study included a higher proportion of heavily treated patients, with 16.9% of the patients undergoing more than three lines of systemic therapies, our study demonstrated substantially improved clinical outcomes in terms of ORR (35%) and median OS (23.8 months). Furthermore, when GX-188E was combined with pembrolizumab, it exhibited favorable ORR and

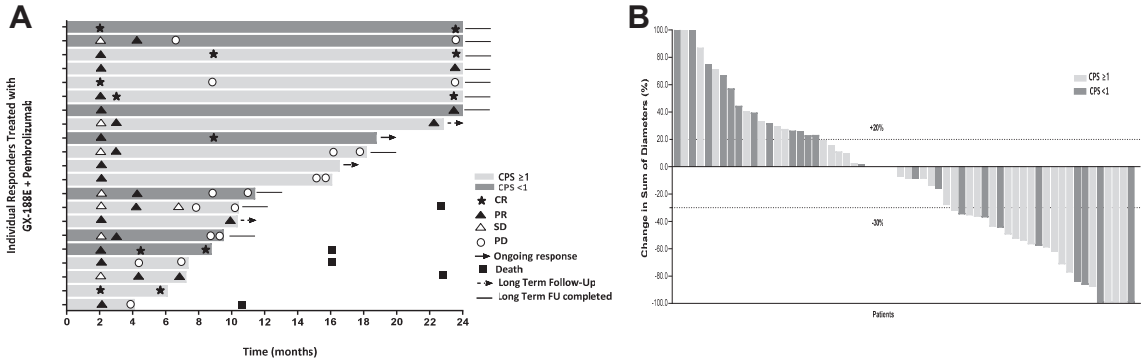

***Fig. 2:* Antitumor activity**. (A) Swimmer's plot of duration of response (DOR) for the patients with 7 complete responses and 14 partial responses after treatment with GX-188E and pembrolizumab. Each bar represents a patient. The star or triangle closest to the Y-axis indicates the first response. Light gray bars indicate PD-L1 positive patients (CPS ≥1), and the dark gray bars indicate PD-L1 negative patients (CPS < 1). (B) Best change from baseline in target lesion size among the total efficacy evaluable population (60 patients) assessed by RECIST v1.1. **Abbreviations:** DOR, Duration of response; PD-L1, Programmed death ligand 1; CPS, Combined positive score; RECIST, the Response Evaluation Criteria in Solid Tumors; CR, Complete response; PR, Partial response; SD, Stable disease; PD, Progression of disease; FU, follow up.

longer median OS compared to existing anti-cancer treatments. According to previous studies, the ORR for gemcitabine was 5%,[18] that for pembrolizumab was 14.3%,[10] that for cemiplimab was 16.4%,[11] that for tisotumab vedotin was 17.8%,[19] and that for cadonilimab was 33%. The respective median OS durations for each therapy were 6.5 months, 9.4 months, 12.0 months, 11.5 months, and 17.5 months. In a recent study, various combination therapies incorporating immunotherapy, including anti-CTLA4 with anti-PD-1/PD-L1 and anti-TIGIT with anti-PD-1/PD-L1, showed encouraging results in patients with recurrent and metastatic cervical cancer. One notable example is the treatment involving nivolumab, either alone or in combination with ipilimumab, which demonstrated an ORR of 38.4% and a median OS of 21.6%. Notably, when administered as 1st- and 2nd-line treatments, this combination therapy displayed distinct ORRs of 40.6% and 34.9%, respectively.[20] Furthermore, the ORR for balstilimab with zalifrelimab was reported to be 25.6%,[21] while that for pembrolizumab with vibostolimab ranged between 15% and 23%.[22]

Our study population exhibited a higher prevalence of adenocarcinoma histology among patients with cervical cancer who received pembrolizumab-based therapies (23.1%) compared to the findings of other studies (5.1–18.2%).[23] Adenocarcinoma is typically associated with a more unfavorable prognosis and higher rates of

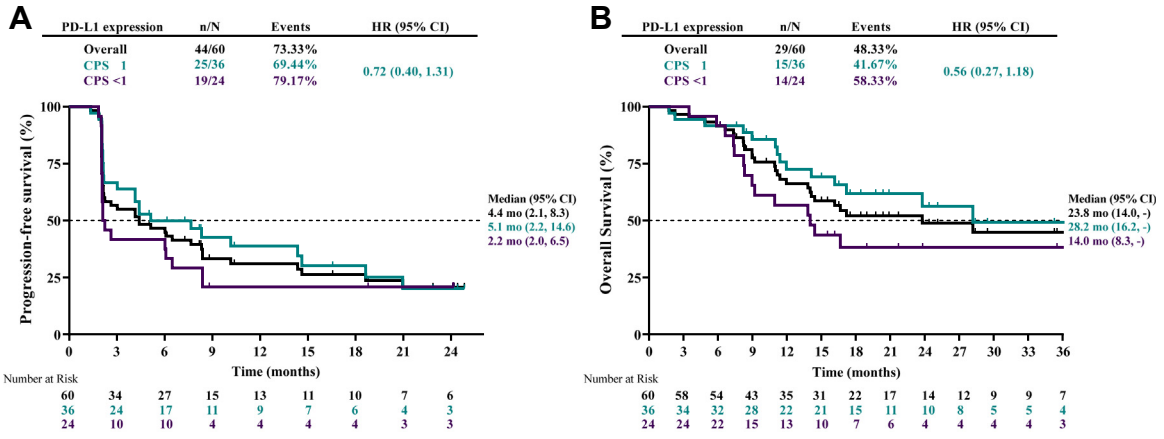

***Fig. 3:* Antitumor activity in the total population**. Kaplan–Meier curves for progression-free survival (A) and overall survival (B) in the overall trial population (black), the population with CPS > 1 (green), and the population with CPS < 1 (purple) are shown. The median PFS and OS are described. Tick marks indicate censored data. **Abbreviations:** PD-L1, Programmed death ligand 1; CPS, Combined positive score; PFS, Progression-free survival; OS, Overall survival; HR, Hazard ratio; mo, months.

Treatment-related adverse events (n = 65)	Grade 1-2	Grade 3	Grade 4	Grade 5
Endocrine disorders	**10 (15.4)**	0	0	0
Hypothyroidism	9 (13.8)	0	0	0
Thyroiditis	1 (1.5)	0	0	0
Gastrointestinal disorders	**6 (9.2)**	**0**	**0**	**0**
Diarrhoea	2 (3.1)	0	0	0
Nausea	2 (3.1)	0	0	0
Vomiting	2 (3.1)	0	0	0
Gastrointestinal pain	1 (1.5)	0	0	0
Stomatitis	1 (1.5)	0	0	0
Skin and subcutaneous tissue disorder	**4 (6.2)**	**0**	**0**	**0**
Pruritus	1 (1.5)	0	0	0
Rash	1 (1.5)	0	0	0
Urticaria	1 (1.5)	0	0	0
Eczema asteatotic	1 (1.5)	0	0	0
Investigations	**3 (4.6)**	**2 (3.1)**	**1 (1.5)**	**0**
Alanine aminotransferase increased	1 (1.5)	0	1 (1.5)	0
Aspartate aminotransferase increased	1 (1.5)	1 (1.5)	0	0
Blood thyroid stimulating hormone increased	2 (3.1)	0	0	0
Tri-iodothyronine increased	1 (1.5)	0	0	0
Neutrophil count decreased	0	1 (1.5)	0	0
Musculoskeletal and connective tissue disorders	**4 (6.2)**	**0**	**0**	**0**
Back pain	1 (1.5)	0	0	0
Myalgia	1 (1.5)	0	0	0
Pain in extremity	1 (1.5)	0	0	0
Joint stiffness	1 (1.5)	0	0	0
Polyarthritis	1 (1.5)	0	0	0
Nervous system disorder	**2 (3.1)**	**1 (1.5)**	**0**	**0**
Syncope	0	1 (1.5)	0	0
Dizziness	1 (1.5)	0	0	0
Peripheral sensory neuropathy	1 (1.5)	0	0	0
General disorders and administration site conditions	**3 (4.6)**	**0**	**0**	**0**
Asthenia	1 (1.5)	0	0	0
Injection site reaction	1 (1.5)	0	0	0
Pyrexia	1 (1.5)	0	0	0
Infections and infestations	**1 (1.5)**	**0**	**0**	**0**
Respiratory, thoracic and mediastinal disorders	**1 (1.5)**	**0**	**0**	**0**
Vascular disorders	**1 (1.5)**	**0**	**0**	**0**
Metabolism and nutrition disorders	**1 (1.5)**	**0**	**0**	**0**

Note: Data are presented as No. (%) unless indicated otherwise.

Table 3: **Treatment-related adverse events that occurred in patients included in the safety analysis.**

distant recurrence when compared to SCC.[23,24] The positive clinical outcomes, including improved OS, observed in this study among patients with a higher prevalence of adenocarcinoma histology types are highly promising.

Our results showed that combining GX-188E and pembrolizumab yielded clinical efficacy regardless of the PD-L1 expression. PD-1/PD-L1 inhibitors that are clinically in use or under trials for recurrent, advanced cervical cancer are mostly effective for tumors expressing PD-L1 (CPS ≥ 1).[10,17] Combination therapies of anti-PD-1/PD-L1 with other therapeutic means have been used to overcome this limitation and enhance the efficacy.[21] In this study, the proportion of patients with CPS ≥ 1 was 60.0%, comparable to that in some previous studies (ranges, 56.8–88.6%).[11,21] The ORR for all the patients, those with CPS < 1, and those with CPS ≥1 was 35% (21/60), 29.2% (7/24) and 38.9% (14/36), respectively. The responses were consistent between the CPS < 1 and CPS ≥ 1 groups (Appendix 2). The tumor microenvironment (TME) is enriched with chemokines, cytokines, and immune modulators secreted by both tumor and stromal cells and plays a critical role in HPV-related tumorigenesis.[25,26] Previous studies suggested

that converting "immune-cold" tumors into "immune-hot" ones could enhance the sensitivity of the HPV-infected TMEs to immunotherapies.[27,28]

A biomarker is important for evaluating treatment response or fitting the treatment effects. The explorative immunological analysis revealed that the differences in IFN-gamma (γ) levels between antigen-specific responses at 4 weeks after the first injection and screening exhibited statistical significance among responders and non-responders. Nevertheless, the increase in antigen-specific T-cell responses was observed regardless of PD-L1 expression ($p > 0.05$, Mann–Whitney U test). This implies that the combination therapy can benefit all patients regardless of the PD-L1 status. Our study showed a significant increase in IFN-γ levels among the responders who received the combination therapy of GX-188E and pembrolizumab. In a previous study, the responders who underwent GX-188E monotherapy consistently demonstrated a substantial increase in IFN-γ serum levels.[29] This finding may suggest that high IFN-γ levels are associated with better response rates in combination therapy and potentially serve as a prognostic biomarker.

The combination therapy was associated with minimal adverse effects. Patients who underwent this therapy experienced thyroiditis and colitis, consistent with the side effects known to be associated with PD-L1/PD-1 inhibitors. However, common side effects of conventional chemotherapy, such as anemia, fatigue, diarrhea, and peripheral neuropathy, were not significantly observed in this study.[10,11] In this study, side effects related to GX-188E injection with electroporation were observed, with local and transient pain, mostly well-tolerated with a short duration, as the primary side effect. This finding aligns with those of previous studies.[13,15]

Recent trials with novel agents as the second-line systemic therapy have resulted in an improvement in response rates for patients with cervical cancer.[10,11,19] In 2018, the FDA approved pembrolizumab based on the Keynote-158 study for patients with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy whose tumors express PD-L1 (CPS ≥1).[10] However, there were no responses in PD-L1-negative patients, preventing its approval in patients with CPS < 1. In 2021, the FDA also approved tisotumab vedotin-tftv for adult patients with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy with 24% ORR, regardless of the PD-L1 expression. Most recently, cadonilimab was approved in China in June 2022 for use in patients with relapsed or metastatic cervical cancer that had progressed on or after platinum-based chemotherapy with 33% ORR, regardless of PD-L1 expression. Although these therapies have improved the median OSs to >1 year (ranging from 12.0 to 17.5 months) compared to the conventional chemotherapeutic agents,[10,11,19,30] novel effective therapies with modest response rates and longer OSs are still needed.

This study had several limitations. First, we did not perform an indirect cross-trial comparison between patients treated with the GX-188E and pembrolizumab combination therapy and those treated with pembrolizumab alone. Second, exploratory analyses of the tumor microenvironment concerning HPV status were not conducted and warrant further investigation. Additionally, the global efficacy and safety of the combination therapy should be explored in broader patient populations across various geographical regions. Despite these limitations, our findings provide valuable insights into the potential of combination therapies in the treatment of cervical cancer and offer a foundation for future research endeavors in this field.

In summary, the combination therapy of GX-188E and pembrolizumab was safe, showed an extended overall survival, and yielded a substantially improved clinical outcome (ORR, 35%). Furthermore, it demonstrated promising effectiveness against tumors irrespective of PD-L1 expressions, indicating its potential as a therapeutic agent targeting oncogenic HPV genes. Given the lack of effective second-line therapies for recurrent, advanced cervical cancer, the combination therapy is a promising novel regimen.

Contributors

JWW, YCS, and JSP contributed to the design of the study. MCL, SYH, YMK, JHN, BGK, CHC, SHK, DHJ, JKL, and JSP contributed to the collection, analysis, and interpretation of clinical data. MCL, YJC, JHK, YC, JWW, YCS, and JSP wrote the first draft of the manuscript and edited the manuscript. YJC and YC verified the underlying data. All authors read and approved the final version of the manuscript. MCL and YJC contributed equally to this work.

Data sharing statement

Qualified researchers may request access to individual patient level data.

The deidentified data that support the findings of this study will be available upon request after publication. To gain access, data requestors will need to sign a data access agreement. Requests can be made to the corresponding author by email.

Declaration of interests

JSP and YCS participated in the scientific advisory board for Genexine. JWW and YCS are stockholders of Genexine, and JWW, YCS, and JSP have a patent PCT/KR2021/005237 issued. YCS is the founder of Genexine. YC and JWW received a grant from the National Cancer Center Onco-Innovation Unit in Korea, and non-financial support from Merck Sharp & Dohme LLC during the conduct of the study. YC is an employee of Genexine. MCL, YJC, SYH, YMK, JHN, BGK, CHC, SHK, DHJ, JKL, and JHK declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2024.102716.

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