

# A Phase II, Prospective, Randomized, Multicenter, Open-Label Study of GX-188E, an HPV DNA Vaccine, in Patients with Cervical Intraepithelial Neoplasia 3

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## ABSTRACT

**Purpose:** To determine the efficacy of the therapeutic DNA vaccine GX-188E for inducing regression of cervical intraepithelial neoplasia (CIN) 3.

**Patients and Methods:** We conducted a prospective, randomized, multicenter, open-label, phase II clinical trial of GX-188E in CIN3 patients positive for human papillomavirus (HPV) type 16/18. The primary endpoint was to determine the histopathologic regression to  $\leq$ CIN1 at visit seven (V7; 20 weeks after the first GX-188E injection), and an extension study was pursued until visit 8 (V8; 36 weeks after the first GX-188E injection). HPV-sequencing analysis and an *ex vivo* IFN $\gamma$  ELISpot assay were performed using the collected cervical biopsy and blood samples from patients.

**Results:** In total, 72 patients were enrolled and underwent randomization. Of them, 64 patients were included in per-

protocol analysis (V7) and 52 in extension analysis (V8). Our data showed 52% (33/64) of patients at V7 and 67% (35/52) of patients at V8 presented histopathologic regression after receiving the GX-188E injection. We found that 73% (V7) and 77% (V8) of the patients with histologic regression showed HPV clearance. HPV clearance and histopathologic regression were significantly associated at V7 and at V8. Compared with the measurements at V1 (baseline), the patients at V8 with HPV clearance showed significantly higher fold changes in their IFN $\gamma$  ELISpot responses compared with those without HPV clearance. The HPV sequence analysis revealed that the HPV type 16 E6/E7 variants D25E, V83L, and N29S were inversely associated with histopathologic regression at V8.

**Conclusions:** GX-188E is an effective therapeutic vaccine against a cohort containing only CIN3 patients.

## Introduction

Cervical cancer is the fourth most common cancer in women (1) and is the second leading cause of cancer-related deaths in young women (20–39 years of age; ref. 2). Persistent infection of high-risk human papillomavirus (HPV) is known to be the sole cause of cervical cancer, with 70% of persistent infections attributed to HPV types 16 and 18 (3). The lack of HPV-specific T-cell immunity induces persistent infection and further progression to cancer (4).

Carcinogenesis is a multi-step process; after HPV infection, normal epithelial cells transform to cervical intraepithelial neoplasia (CIN) grades 1, 2, and 3 and then to cervical cancer (Bethesda classification system; refs. 5, 6). CIN3 is a premalignant lesion, and only 0%–26% of CIN3 with HPV16 infections regress spontaneously (4, 7, 8). If CIN3 is not treated, >30% of the patients progress to cervical cancer, whereas only 0.7% progress with treatment (9, 10). Therefore, it is necessary to treat CIN3 to hinder its progression to cancer. The current standardized treatment for CIN3 is surgical removal of the lesion; no effective nonsurgical treatments have been approved (10). However, surgical treatment increases adverse pregnancy outcomes, such as premature birth and premature membrane rupture. Because the incidence of cervical cancer in young women is increasing (2), the development of nonsurgical treatments for CIN3 is paramount.

From a decade ago, the therapeutic HPV DNA vaccines to treat cervical premalignant lesions that target E6/E7 have been developed and have shown effective antitumor activity (11, 12). However, more research is necessary to meaningfully evaluate clinical efficacy. GX-188E is a HPV E6/E7 DNA therapeutic vaccine (Genexine, Inc.), consisting of a tissue plasminogen activator signal sequence, an FMS-like tyrosine kinase 3 ligand, and shuffled E6 and E7 genes of HPV type 16/18, as described previously (13). In a phase I study, women with CIN3 were immunized with the GX-188E DNA vaccine by electro-poration, and 78% (7/9 patients) of them presented complete regression of the cervical lesion and viral clearance within 36 weeks after drug administration (13). This phase II clinical trial was designed to test the effect of GX-188E on suppressing persistent HPV infections and regression of HPV-induced cervical intraepithelial lesions in a larger population than that of the phase I clinical trial. The aims were (i) to assess efficacy of GX-188E in patients with CIN3 using the histopathologic results of cervical biopsy and (ii) determine the optimal dose of GX-188E (GX-188E 1 or 4 mg).

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### Translational Relevance

In this study, we report that a human papillomavirus (HPV) DNA vaccine (GX-188E) demonstrates the efficacy in cervical intraepithelial neoplasia 3 (CIN3) patients with HPV16/18. Among 72 patients who were enrolled and underwent randomization, 52% at visit seven (V7; 20 weeks after the first injection) and 67% at visit eight (V8; 36 weeks after the first injection) presented histopathologic regression after receiving the GX-188E injection, indicating a clinical benefit of the HPV DNA vaccine for treating CIN3. Because HPV E6 and E7 variants were inversely associated with histologic regression, HPV sequences of each patient should be considered to design individualized HPV DNA vaccines.

## Patients and Methods

### Study design and patients

This study was a prospective, randomized, multicenter, open-label, phase II trial conducted at four Korean sites: the Catholic University of Seoul St. Mary's Hospital (Seoul, South Korea), the Cheil Hospital (Seoul, South Korea), the Korea University Guro Hospital (Seoul, South Korea), and the Keimyung University and Dongsan Hospital (Daegu, South Korea). This trial is registered at ClinicalTrials.gov (no. NCT02139267). The protocol was approved by the institutional review board or ethics committee at each study site, and a written informed consent was obtained from each patient. The study was conducted in accordance with the Declaration of Helsinki and all applicable laws. The full details of the study design are described in Fig. 1. The efficacy analyses were performed using data collected on the per-protocol group, which was comprised of the patients who completed the study without major violations (e.g., violations of inclusion/exclusion criteria and violations of compliance). Safety analyses were performed using data collected from the safety group, which includes patients who were randomized and received at least one GX-188E injection (71 patients). Adverse events (AE) were monitored for 20 weeks after receiving the treatment.

Inclusion criteria included female patients 19–50 years old that were histopathologically diagnosed with CIN3 from an HPV type 16/18 (+) infection. The histopathology of each sample was reviewed by the central laboratory at the Cheil Hospital. Women were excluded from the study if pregnant, breastfeeding, immunodeficient (e.g., patients with class C hepatic impairment, patients with positive serum test results for human immunodeficiency virus, etc.), suspected of having *in situ* adenocarcinoma within the lesion, having another malignant tumor, or having any other condition at the discretion of the principal investigator.

### Randomization

At the randomization visit, each eligible patient was randomly assigned (1:1) to two different dose groups (1 or 4 mg) of GX-188E. Before randomization, it was confirmed that the patient was diagnosed with CIN3 from an HPV type 16/18 (+) infection.

### Procedures

Patients were randomly assigned to treatment groups and received either 1 or 4 mg of GX-188E intramuscularly by electroporation (TriGrid Delivery System, Ichor Medical Systems, Inc.) in the deltoid muscle. Drug administration was performed three times in total during

the study period at visits (V) 2, 3, and 5 (weeks 0, 4, and 12, respectively) with sequential doses applied to the alternate arm compared with the previous visit. Follow-up visits (6 and 7) occurred 14 and 20 weeks after the initial GX-188E administration (Fig. 1A). At V7, the efficacy of GX-188E was evaluated by a colposcopy-directed cervical biopsy and an HPV DNA test [Seeplex HPV4A ACE Screening (Seegene)]. The patients who completed the 20-week study were provided the option of entering the extension study. In the extension phase, patients were reevaluated at visit 8 (V8; 36 weeks after the first GX-188E injection) by a colposcopy-directed cervical biopsy (Fig. 1B).

DNA was extracted from the cervical biopsies and nucleotide sequencing was performed to confirm all patients were positive for HPV type 16. The resulting HPV sequences were compared with the published HPV 16 type E6 and E7 amino acid sequences (GenBank accession no. AAL96630 and NP041326, respectively). HPV16 E6/E7 sequences were amplified by PCR using the long-fragment primers, 5'-AAA CTA AGG GCG TAA CCG AAA-3' and 5'-CGC ATG TGC TGT CTC TGT T-3'. If the long-fragment PCR was unsuccessful, short-fragment PCR was performed using primer pairs 5'-GTT CTG CTT GTC CAG CTG GA-3' and 5'-TCA AAA GCC ACT GTG TCC TG-3'.

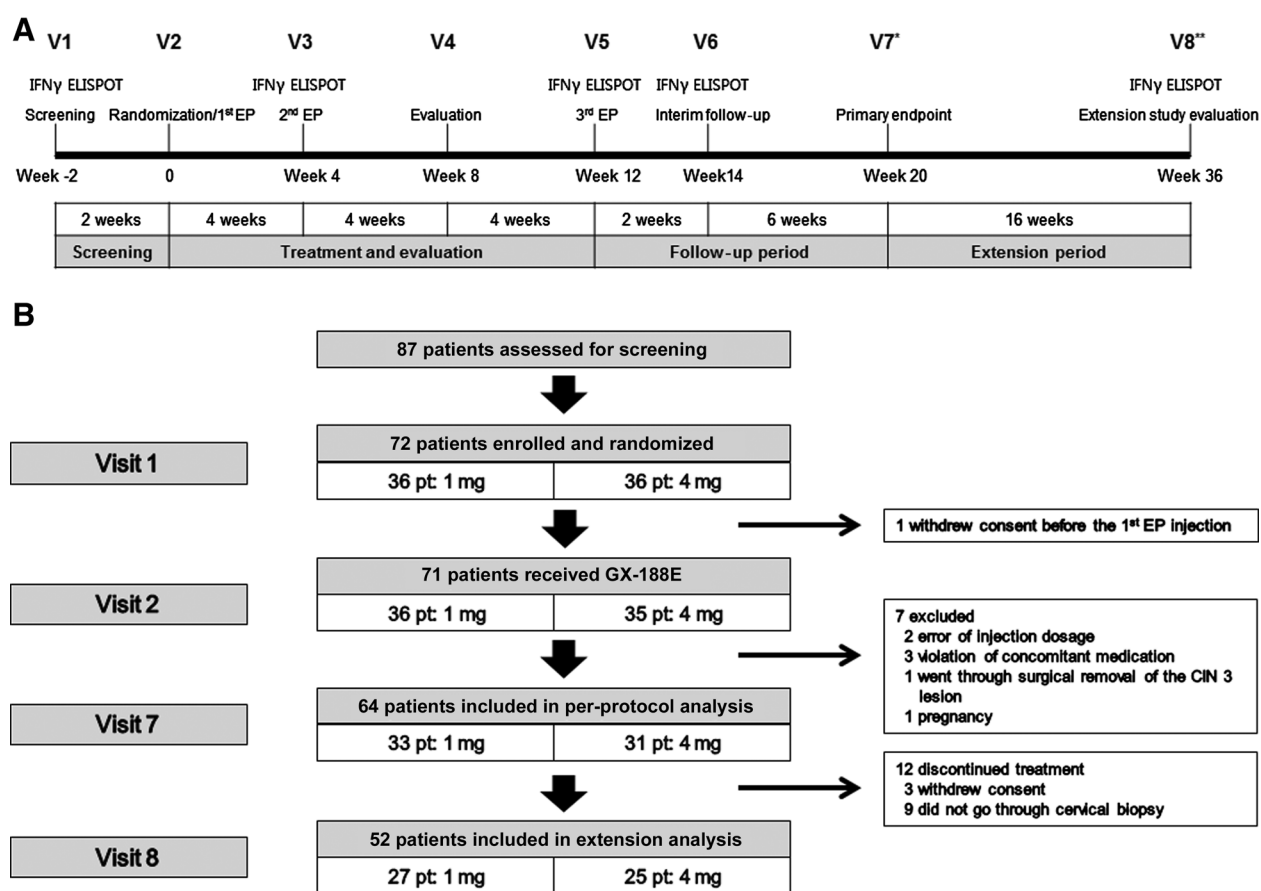
To investigate the cellular immune response induced by GX-188E, the HPV type 16/18 E6/E7-specific T-cell responses (*ex vivo* IFN $\gamma$  ELISpot) were analyzed at V2, V3, V5, V7, and V8 using whole-blood sample (Fig. 1). For the *ex vivo* IFN $\gamma$  ELISpot analysis (BD Bioscience), cryopreserved and thawed peripheral blood mononuclear cells (PBMC) were adapted with Op Tmizer CTS Medium (Life Technologies) and were stimulated with four different pools of HPV16 and HPV18 E6- or E8-derived peptides (20-mer with 10 amino acids overlapping) for 48 hours. The process in detail is described in the phase I study (13). IFN $\gamma$  ELISpot responses to HPV E6/E7 were determined by comparing signals with the baseline levels measured prior to vaccination (V8/V1) and the average sums of the IFN $\gamma$  ELISpot responses, as calculated by: [average (V3 – V8)]/V1. IFN $\gamma$  ELISpot responses  $\geq 3$ -fold over baseline indicated the drug was efficacious.

The safety of the investigational product was evaluated by recording, reporting, and analyzing the results of the laboratory tests and physical examination findings, which considered the patient's underlying disease, adverse reactions, and signs of vitality. A comprehensive evaluation of AEs experienced by patients, such as drug toxicity, was conducted. The investigators evaluated the severities of the reactions based on the Common Terminology Criteria for Adverse Events (CTCAE v4.03) by the NCI (NIH).

Human leukocyte antigen (HLA) typing was accomplished at the Catholic Hematopoietic Stem Cell Bank, College of Medicine, the Catholic University of Korea (Seoul, Korea). The methods have been described previously (13).

### Outcomes

The primary endpoint was to determine the histopathologic regression to  $\leq$ CIN1 at V7 (20 weeks after the first GX-188E injection) among the HPV type 16/18 (+) CIN3 patients. Patients were considered as regressors when the principal investigator considered the colposcopic findings as so ( $n = 4$  at V8). The patients were considered to be “nonregressors” if the cervical tissues collected at each evaluation visit [V7 (primary endpoint) or V8 (extension phase; 36 weeks after the first GX-188E injection)] were  $\geq$ CIN2. The nonregressors group also included patients who underwent surgery (e.g., loop electrosurgical excision) before V7 or V8 due to a lack of regression. The secondary efficacy outcome was calculated as the proportion of patients with



**Figure 1.** Study design. **A**, Visit schedule. †, V7: within 20 weeks of the first GX-188E injection. ††, V8: within 36 weeks of the first GX-188E injection. **B**, Clinical trial profile. EP, electroporation.

HPV viral clearance at V7 and V8. Safety was analyzed for all patients receiving at least one dose of GX-188E injection and the mean visual analogue scale was used to measure pain at each administration.

### Statistical analysis

The planned efficacy evaluation experiments required 64 patients (32 patients in each treatment group); accounting for 10% attrition, a total of 72 subjects were recruited. The Bayesian Pick-the-Winner method (or SWE method) proposed by Simon and colleagues was used to randomly assign patients to the two treatment groups. The numbers of required subjects were calculated following the detailed methodology described in the Supplementary Data.  $\chi^2$  comparisons and *t* tests were performed to determine statistical significances of all quantitative data using the Statistical Package for the Social Sciences v24.0. All *P* values less than 0.05 were considered significant.

## Results

### Patient disposition and baseline characteristics

Of the 87 patients screened, 72 were randomized, and 71 were exposed to either 1 mg ( $n = 36$ ) or 4 mg ( $n = 35$ ) GX-188E (Fig. 1; Supplementary Table S1). Table 1 summarizes the characteristics of the patients who received GX-188E by electroporation. CIN3 lesions

were subclassified according to cervical lesion size (lesions that cover <50% vs. >50% of the cervix by colposcopy). There were no statistically significant differences in age and cervical lesion size between the groups who received the 1 and 4 mg doses of GX-188E.

### Outcomes

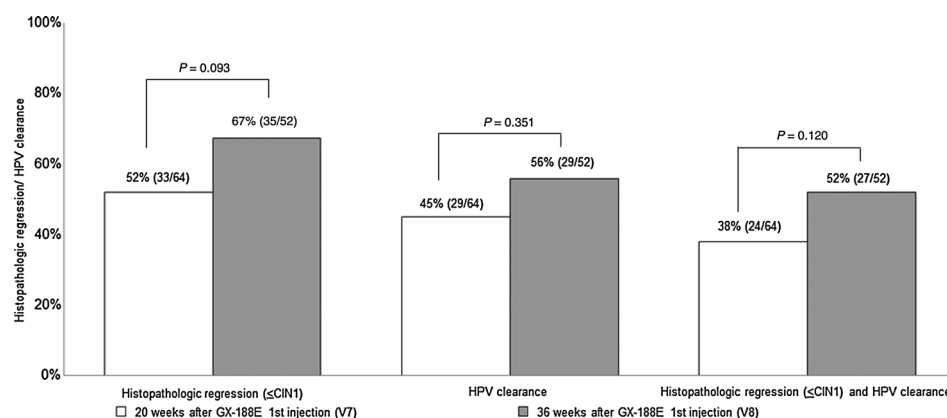
Of 72 screened patients, 64 were included in the per-protocol group who participated in the study until 20 weeks after the first GX-188E injection (V7). Histopathologic regression occurred in 33 of the 64 patients (Fig. 2). Of the 64 patients in per-protocol group, 52 were included in the extension study to be evaluated at 36 weeks (V8) after the first GX-188E injection (Fig. 1). The overall efficacy was higher at the V8 (35/52 patients) than the V7 evaluation based on histopathologic regression. We subclassified the CIN3 according to the cervical

**Table 1.** Characteristics of patients with CIN3.

	1 mg ( <i>n</i> = 36)	4 mg ( <i>n</i> = 35)	Total ( <i>n</i> = 71)	<i>P</i>
Age (years), mean (SD)	33.4 (4.6)	31.4 (6.1)	32.2 (5.4)	0.343
Cervical lesion size				0.089
<50%	15 (41.7%)	21 (58.3%)	36	
>50%	21 (60.0%)	14 (40.0%)	35	

**Figure 2.**

Clinical efficacy. The percentages of patients with histopathologic regression (<CIN1), HPV clearance, and concomitant histopathologic regression (<CIN1) and HPV clearance at V7 and V8. The statistical analysis was done with  $\chi^2$  test.



lesion size (lesions that cover <50% vs. >50% of the cervix by colposcopy) and found that the ones <50% showed better efficacy than the ones >50% after GX-188E injection. Of 32 patients with cervical lesion size <50%, 63% (20/32) showed histopathologic regression, whereas only 41% (13/32) in the group presented with cervical lesion size >50% (V7;  $\chi^2$  test;  $P = 0.133$ ). At V8, 83% (24/29) showed histopathologic regression among the patients with cervical lesion size <50%, whereas only 48% in the group presented with cervical lesion size >50% ( $\chi^2$  test;  $P = 0.016$ ). We found that the patients with cervical lesion size <50% presented higher histopathologic regression rate than the ones with cervical lesion size >50% with statistical significance (Supplementary Table S1). In addition, the number of patients with HPV clearance and histopathologic regression with HPV clearance were found to have increased when V7 was compared with V8 (Fig. 2).

An IFN $\gamma$  ELISpot assay was performed at V1 (baseline, before injection) and V8 (36 weeks after the initial dose) using PBMCs from 47 of the 64 patients in the per-protocol group (Supplementary Table S2). As described in Materials and Methods section, because cryopreserved and thawed PBMCs were used in this assay, we could obtain viable PBMCs from only 47 patients in both V1 and V8. Of them, 7 of the 22 patients without histopathologic regression and 16 of the 25 patients with histopathologic regression exhibited marked increases ( $\geq 3$ -fold) in IFN $\gamma$  ELISpot responses to HPV E6/E7 compared with the baseline level prior to vaccination (V8/V1; Fig. 3). Thus, a significantly higher percentage of the patients with histopathologic regression exhibited marked increases ( $\geq 3$ -fold increase) in IFN $\gamma$  ELISpot responses compared with the group without histopathologic regression ( $\chi^2$  test;  $P = 0.028$ ).

#### HPV clearance and GX-188E treatment efficacy

We next analyzed whether HPV clearance was associated with the efficacy of GX-188E, the HPV E6/E7 DNA therapeutic vaccine. Of the patients with histopathologic regression, 73% (24/33) exhibited HPV clearance at V7 and 77% (27/35) exhibited clearance at V8. Of the nonregressors, 16% (5/31) exhibited HPV clearance at V7 and 12% (2/17) exhibited clearance at V8 (Supplementary Fig. S1 and Supplementary Table S1). We found that HPV clearance and histopathologic regression were significantly associated at the V7 [OR = 13.867; 95% confidence interval (CI), 4.070–47.249;  $P < 0.001$ ] and V8 visits (OR = 25.313; 95% CI, 4.750–14.883;  $P < 0.001$ ).

In addition, we investigated whether HPV clearance was associated with IFN $\gamma$  ELISpot responses. IFN $\gamma$  ELISpot responses to HPV E6/E7 at V8 were compared with the baseline level (V1) prior to vaccination (V8/V1). We found that the patients with HPV clearance ( $n = 26$ ) presented statistically significant increases in IFN $\gamma$  ELISpot responses

compared with those without clearance ( $n = 21$ ; fold changes were 28 and 10, respectively;  $t$  test;  $P = 0.002$ ).

#### HPV sequence variants and GX-188E treatment efficacy

Next, we evaluated whether HPV sequence variations and histopathologic regression at V8 (extension study, 36 weeks after the first GX-188E injection) were associated. Of the 52 cervical tissue samples obtained at V8, 42 were analyzed and 10 nonsynonymous variations were observed (nine E6 variants and one E7 variant; Table 2; Supplementary Table S3). Of them, H14Q (T145G) was detected in most of the cervical tissues, but no significant differences were noted among “nonregressors” and “regressors.” We also found that D25E (T178G), V83L (G350T), and N29S (A647G) were negatively associated with histopathologic regression at V8 (Table 2). Of the 42 tested samples, 26 harbored at least one of the D25E (T178G), V83L (G350T), and N29S (A647G) variants, and 16 harbored none. We found that histopathologic regression occurred in 42% (11/26) of the CIN3 patients with HPV variants [containing at least one of D25E (T178G), V83L (G350T), and N29S (A647G)], whereas 75% (12/16) occurred in those without any of the three variants (Fig. 4A).

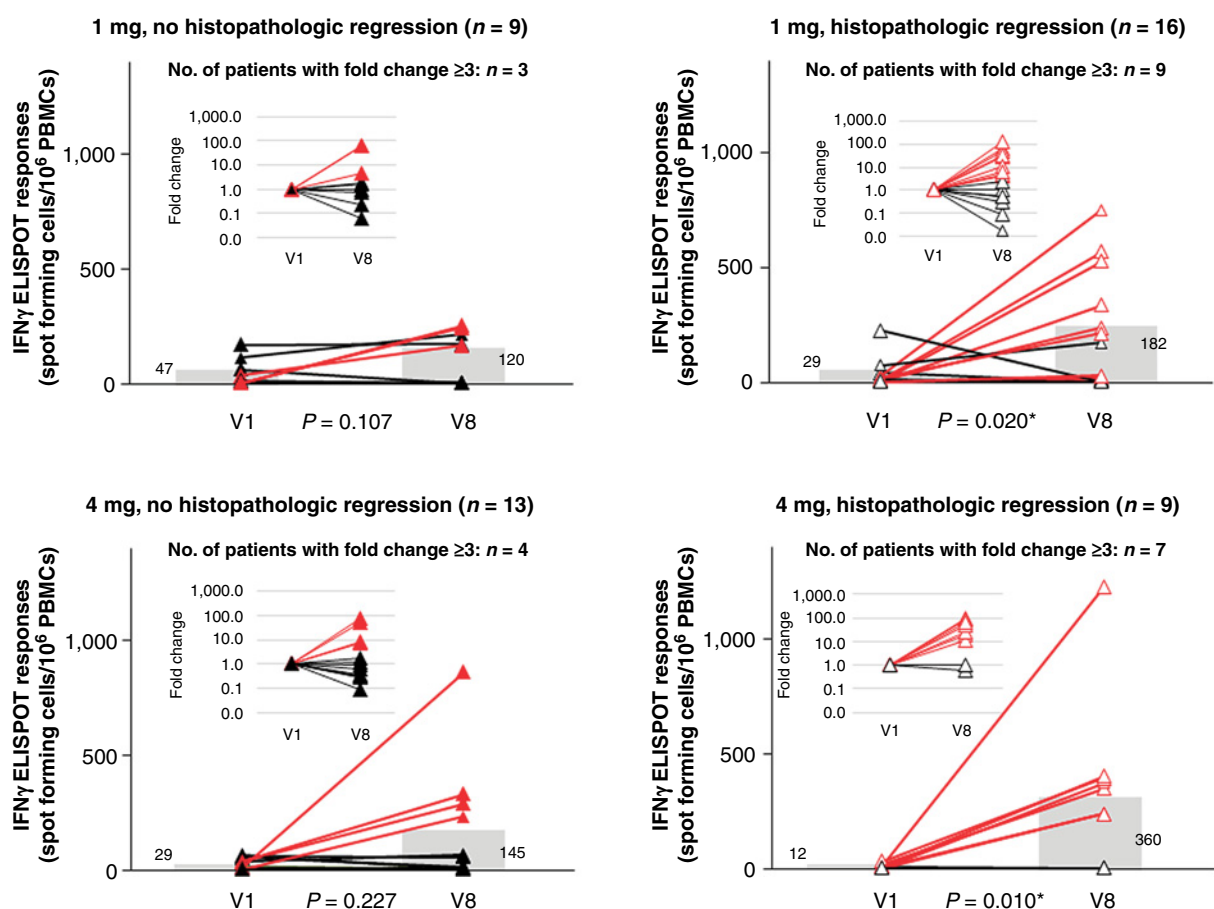
Next, we sought to determine why less histopathologic regression occurred in patients with the HPV variations by analyzing the association between the fold changes of the IFN $\gamma$  ELISpot responses [(average(V3 – V8))/V1] and the HPV variants. We found that CIN3 patients with the D25E (T178G) and N29S (A647G) variants were associated with lower IFN $\gamma$  ELISpot fold changes ( $t$  test;  $P = 0.005$  and  $P = 0.003$ , respectively) after GX-188E injection (1 or 4 mg). The patients with V83L (G350T) also showed lower IFN $\gamma$  ELISpot fold changes after treatment; although it was not statistically significant (Fig. 4B; Supplementary Table S4).

#### GX-188E 1 versus 4 mg

When the efficacies were compared between the 1 and 4 mg GX-188E groups (Supplementary Fig. S2), 1 mg was found to have better efficacy in terms of histopathologic regression, HPV clearance, and histopathologic regression with HPV clearance at V7 and V8. In addition, we found that HPV clearance and histopathologic regression, and HPV clearance with the 1 mg dose of GX-188E were significantly increased compared with the group that received the 4 mg dose ( $\chi^2$  test;  $P = 0.006$  and  $P = 0.027$ , respectively) at V8.

#### HLA types

In addition, we evaluated whether HLA types were associated with histopathologic regression and HPV clearance. We found that HLA-



**Figure 3.**

Induction of HPV-specific IFN $\gamma$  responses induced by the GX-188E vaccination as determined by an ELISpot assay. HPV16/18 E6 and E7 ELISpot responses (spot forming unit per  $10^5$  PBMCs) for each patient at baseline (V1) and at 36 weeks after the first GX-188E injection (V8) were calculated (V8/V1). PBMCs were isolated from the patients' blood and cryopreserved before and after vaccination. Numbers of the fold changes that increased more than three are written, and the graph is shown in red. The gray boxes indicate the "average" sums of the IFN $\gamma$  ELISpot responses. IFN $\gamma$  ELISpot responses at the baseline (V1) and those at the time of histologic analysis (V8) were significantly different (*t* tests;  $P = 0.02$  in 1 mg;  $P = 0.01$  in 4 mg, respectively) in patients with histopathologic regression. In contrast, there is no significant difference in IFN $\gamma$  ELISpot responses between V1 and V8 in patients with no histopathologic regression (*t* tests;  $P > 0.05$ ). \*, statistically significant associations (*t* tests;  $P < 0.05$ ).

A\*02 was associated with histopathologic regression at V7 (20 weeks after the first injection;  $P = 0.032$ ; OR = 2.381; 95% CI, 1.064–5.327), but not at V8 (36 weeks after the first injection;  $P = 0.404$ ; OR = 1.490; 95% CI, 0.582–3.811; Supplementary Table S1).

#### AEs

The safety group consisted of 71 patients, excluding the 1 patient who did not receive GX-188E after randomization because of their unwillingness to continue with the study (Table 3). GX-188E was well-tolerated by all the patients. The numbers of AEs among the two groups (GX-188E 1 and 4 mg) were similar. The AEs relating to the injection site were pain, erythema, induration, and swelling/edema in both groups; pain was the most common AE (occurring in 94.4% and 100.0% in the 1 and 4 mg GX-188E groups, respectively). Average duration of injection site–related AE is 1.98 days for 1 mg group and 2.12 days for 4 mg group, respectively. The incidence of serious AEs was 5.56% among patients who received the 1 mg dose and 2.86% among patients who received the 4 mg dose; however, none of these events were found to be related to either the DNA vaccine or

electroporation device. Two serious AEs were pneumonia (1 in each group) and one was pregnancy (1 mg GX-188E group). The patient with pregnancy was the only one to discontinue participation in the study.

#### Discussion

This prospective, randomized, multicenter, open-label, phase II clinical trial tested the efficacy of the HPV DNA therapeutic vaccine, GX-188E, in CIN3 patients with HPV type 16/18. It was found that the vaccine resulted in 52% and 67% histopathologic regression at V7 (20 weeks after the first injection) and V8 (36 weeks after the first injection), respectively. We also found that HPV variants that are known to affect HPV persistence and cervical cancer progression were inversely associated with GX-188E–induced clinical outcomes. CIN3 patients with the HPV variants, D25E (T178G), V83L (G350T), and N29S (A647G), showed less histopathologic regression (42%) compared with those without those variants (75%).

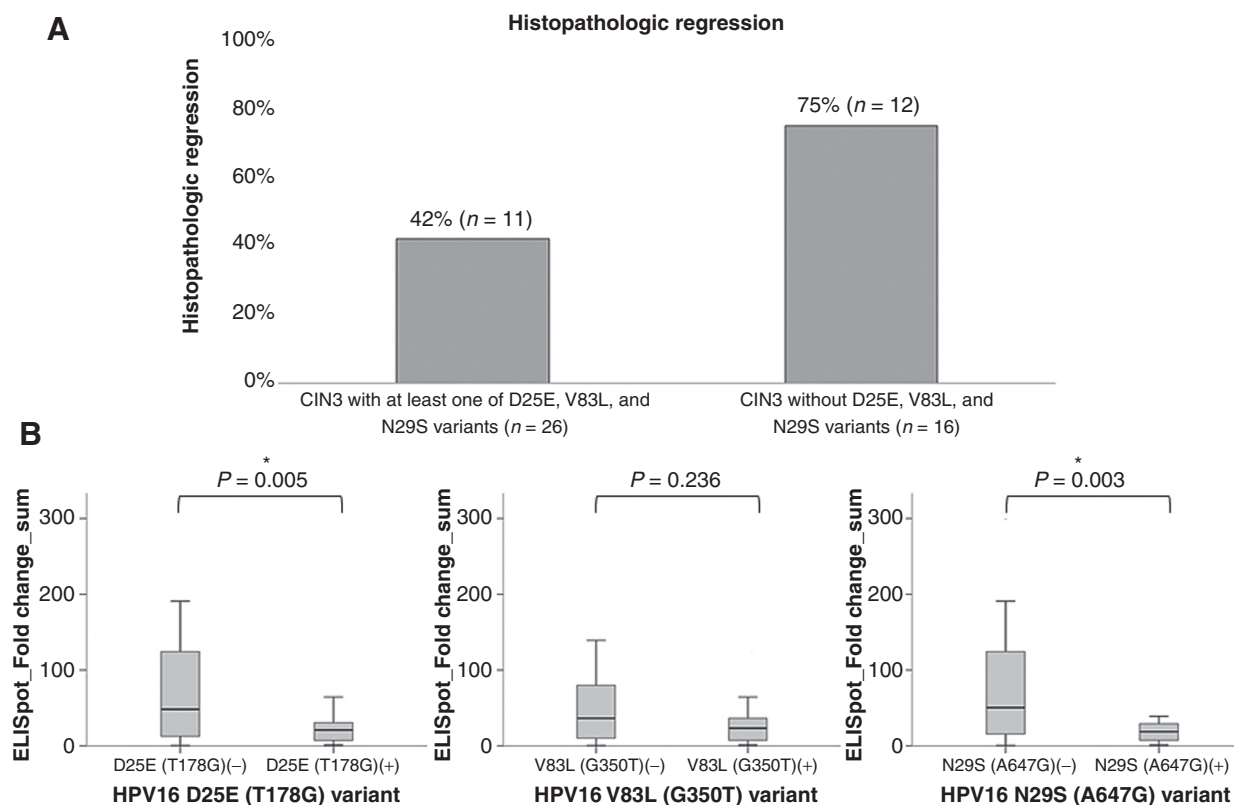
**Table 2.** List of HPV16 single-nucleotide variations among the patients included at V8 visit.

HPV variation	Nonregressor (n = 14/17 patients)	Regressor (n = 28/35 patients)	P	OR (95% CI)
Q5H (A90C)	1	0	0.333	0.317 (0.202–0.497)
R3I (G96T)	0	1	1.000	0.659 (0.528–0.821)
H14Q (T145G)	13	17	0.014	0.119 (0.014–1.042)
D25E (T178G) <sup>a</sup>	11	11	0.016	0.176 (0.040–0.779)
I27R (T183G)	1	3	1.000	1.560 (0.147–16.527)
Y78H (T335C)	13	18	0.047	0.138 (0.016–1.220)
V83L (G350T) <sup>a</sup>	12	12	0.008	0.125 (0.023–0.666)
E113D (A442C)	2	2	0.590	0.462 (0.058–3.679)
R141T (G525C)	1	0	1.333	0.317 (0.202–0.497)
N29S (A647G) <sup>a</sup>	12	11	0.004	0.108 (0.202–0.578)

<sup>a</sup>HPV variants significantly associated with histopathologic regression ( $P < 0.05$ ).

To our knowledge, GX-188E is the most effective therapeutic vaccine to yield histopathologic regression in CIN3 with HPV16 (+) patients. We tested two doses (1 and 4 mg) and found that the 1 mg dose had better efficacy, which may indicate hormesis (an inverted U-shaped dose–response relationship; ref. 14). Among all of the participants, 52% showed histopathologic regression at 20 weeks after the first GX-188E injection (at V7). At 36 weeks after the first GX-188E injection (V8), histopathologic regression was observed in 67% of

the patients, and the efficacy increased up to 83% among those with cervical lesions <50% in the subgroup analysis. The increasing rate of histopathologic regression may be because memory T-cell-driven therapeutic effects persist over time. A randomized phase II clinical trial with VGX-3100, a therapeutic vaccine, showed 49.5% efficacy based on histopathologic regression among CIN2/3 patients (12). Another randomized phase II clinical trial with the therapeutic vaccine, TV4001, resulted in a clinical response of 48% in CIN2/3



**Figure 4.** Histopathologic regression and HPV variants [D25E (T178G), V83L (G350T), and N29S (A647G)]. **A**, HPV nucleotide sequencing was performed on 42 CIN3 patient samples. Among them, 26 harbored at least one of the D25E (T178G), V83L (G350T), or N29S (A647G) variants, and 16 did not. We found that histopathologic regression occurred in 42% (11/26) of the CIN3 patients with D25E (T178G), V83L (G350T), and N29S (A647G) variants compared with 75% (12/16) for the group without any of the three variants. **B**, The association between the fold changes of the IFN $\gamma$  ELISpot responses [(average (V3 – V8))/ V1] and the HPV variants. \*, statistically significant associations ( $P < 0.05$ ).

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**Table 3.** AEs that occurred during treatment.

Overall (safety set group)	1 mg (n = 36) No. (%)	4 mg (n = 35) No. (%)
Any AE or injection-site reaction	34 (94.4%)	35 (100.0%)
G1 AE or injection-site reaction	34 (94.4%)	35 (100.0%)
G2 AE or injection-site reaction	31 (86.1%)	33 (94.3%)
G3 AE or injection-site reaction	10 (27.8%)	22 (62.9%)
Serious AE	2 (5.6%)	1 (2.9%)
<b>Systemic</b>		
Nasopharyngitis	8 (22.2%)	5 (14.3%)
Dysmenorrhea	4 (11.1%)	3 (8.6%)
Vaginal infection	3 (8.3%)	5 (14.3%)
Headache	1 (2.8%)	2 (5.7%)
Myalgia	1 (2.8%)	3 (8.6%)
<b>Local injection site</b>		
Pain	34 (94.4%)	35 (100.0%)
Erythema	9 (25.0%)	17 (48.6%)
Induration	8 (22.2%)	17 (48.6%)
Swelling or edema	8 (22.2%)	14 (40.0%)

patients (15). Our results likely differ from previous studies of clinical benefit, because we only recruited patients with CIN3. It is known that the regression rates of CIN2 and CIN3 differ. CIN2 has regression rate of 27%–60% (16–18) and CIN3 has a rate of 2%–31% (8, 18). In addition, spontaneous regression of CIN2/3 together was observed in some studies, which reported a rate of 30.6%–50% (12, 19). Among them, only 0%–26% of CIN3 with HPV16 (+) regress spontaneously (4, 7, 8). Although it is not possible to distinguish vaccine-related histopathologic regression over spontaneous regression, our data showed GX-188E yielded >50% regression in CIN3 with HPV 16 (+) patients and it may suggest a promising nonsurgical therapy for cervical premalignant lesions.

To investigate whether HPV histopathologic regression was associated with GX-188E treatment efficacy, we evaluated the GX-188E-induced systemic immune response using an IFN $\gamma$  ELISpot assay. When IFN $\gamma$  ELISpot responses to HPV E6/E7 at V8 were compared with the baseline level prior to vaccination (V1), we found that the patients with histopathologic regression (n = 25) presented statistically significant increases in IFN $\gamma$  ELISpot responses compared with these without histopathologic regression (n = 22; Fig. 3). In addition, we found 16 of 25 patients with histopathologic regression exhibited marked increases ( $\geq 3$ -fold increase) in their IFN $\gamma$  ELISpot responses with statistical significance, but 7 of 22 nonregressed patients developed more than 3-fold increase in these responses, indicating that these systemic immune responses induced by GX-188E treatment may be associated with histopathologic regression.

However, the ELISpot responses did not perfectly match the histopathologic regression, because some of the patients with systemic immune response did not present histopathologic regression and vice versa. These results are similar to the previous reports that DNA vaccines induced detectable levels of HPV-specific IFN $\gamma$ , which were not correlated with histologic regression of cervical lesions (20, 21). A previous study suggested that local immune response along with systemic immune response result in histologic regression (22). Other studies also showed that a therapeutic HPV DNA vaccination in combination with intravaginal administration of immune modulator recruits antigen-specific CD8<sup>+</sup> T cells to the genital tract, leading to tumor control in a mouse cervical cancer model (23, 24). Thus, further study to investigate a role of local and

systemic immunity in efficacy of therapeutic vaccine in CIN3 patients may be needed.

This study is the first to indicate an association between HPV type 16 E6/E7 nucleotide sequences and the efficacy of a therapeutic vaccine, GX-188E. We found that D25E (T178G), V83L (G350T), and N29S (A647G) were negatively associated with histopathologic regression at V8. All three of these variants are associated with HPV persistence and cervical cancer progression (25, 26). V83L (G350T) belongs to European lineage, and D25E (T178G) and N29S (A647G) belong to Asian lineage (27–29). We found that CIN3 patients with at least one of the three HPV variants showed lower regression rates (42%) and less HPV-specific T-cell responses after receiving the GX-188E vaccine compared with those without (75%) the variants. However, the regression rate after GX-188E was injected into CIN3 patients with one of the three HPV variants was higher than the reported self-regression rate (8, 18).

GX-188E is an effective therapeutic vaccine against HPV type 16/18-associated CIN3 in a phase II clinical trial. However, because there are no control groups in this study, further study to evaluate a therapeutic effect of the GX-188E against HPV type 16/18-associated CIN3 should be addressed in the placebo-controlled clinical trial. In addition, this study is the first to show associations between HPV variants and HPV therapeutic vaccine efficacy. It remains to be evaluated whether immunization with HPV DNA vaccine containing HPV-variant sequences can induce better efficacy than that with GX-188E vaccine used in this study. Also it is suggested that further study to individualize HPV therapeutic vaccine considering individual patient's HPV variants be investigated.

#### Disclosure of Potential Conflicts of Interest

K.S. Park and Y.S. Suh are employees/paid consultants for and hold ownership interest (including patents) in Genexine, Inc. J.W. Woo and J.S. Park are employees/paid consultants for Genexine, Inc. No potential conflicts of interest were disclosed by the other authors.

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