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Protective immunity with an E1 multivalent epitope DNA vaccine against cottontail rabbit papillomavirus (CRPV) infection in an HLA-A2.1 transgenic rabbit model

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Abstract

Cottontail rabbit papillomavirus (CRPV)/rabbit model is widely used to study pathogenesis of papillomavirus infections and malignant tumor progression. Recently, we established HLA-A2.1 transgenic rabbit lines and demonstrated efficacy for the testing of immunogenicity of a well-known A2-restricted epitope (HPV16E7/82–90)[1]. In the present study, we screened five HLA-A2.1 restricted epitopes from CRPV E1 (selected using online MHCI epitope prediction software) and constructed a multivalent epitope DNA vaccine (CRPVE1ep1-5). CRPVE1ep1-5 and a control DNA vaccine (Ub3) were then delivered intracutaneously onto normal and HLA-A2.1 transgenic rabbits respectively by a helium-driven gene-gun delivery system. One, two or three immunizations were given to different groups of animals from both New Zealand White outbred and EIII/JC inbred genetic background. Two and three immunizations with CRPVE1ep1-5 DNA vaccine provided complete protection against viral DNA infection of HLA-A2.1 transgenic rabbits from both genetic backgrounds but not in the control vaccinated groups. One immunization, however, failed to protect HLA-A2.1 transgenic rabbits against viral DNA infection. This study further demonstrated that the HLA-A2.1 transgenic rabbits can be used to test the immunogenicity of HLA-A2.1 restricted epitopes identified by MHCI epitope prediction software.

Keywords

CRPV; E1; Epitope DNA Vaccine; HLA-A2.1; Transgenic Rabbit; vaccination; protective immunity

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Introduction

High-risk human papillomavirus (HPV) infections cause virtually all cases of cervical cancer, the second most common cause of death from cancer among women worldwide[2]. An effective prophylactic vaccine, Gardasil, approved for clinical use has shown a remarkable degree of protection [2,3]. However, this vaccine is not expected to eliminate preexisting HPV disease. Therefore, a therapeutic vaccine against HPV infection would be highly desirable for prevention of cancer-associated complications.

Despite ongoing efforts to develop effective therapeutic vaccines against HPV and other viral infections, none has been shown to be highly effective clinically, probably because the vaccines have yet to adequately mimic critical aspects of a curative immune response [4]. Lack of effective *in vivo* models has also hampered studies on the mechanism of the HPV-associated malignancy. Several *in vivo* animal papillomavirus infection models are available to study viral immunity and pathogenesis. The cottontail rabbit papillomavirus (CRPV)/rabbit model mimics features of high-risk human papillomavirus infection and has been used widely in vaccine development [5]. This model can be used to dissect the mechanism of papillomavirus-induced malignancy and identify immune targets leading to immunotherapeutic clearance of HPV induced cancer.

Our previous studies have demonstrated that a newly established HLA-A2.1 transgenic rabbit model generated a strong immune response to a well-known HLA-A2.1 restricted epitope from HPV16 E7 *in vivo*[1]. In this paper, we extend these studies to test the immunogenicity of some naturally existing HLA-A2.1 restricted epitopes in CRPVE1. E1 is an early papillomavirus protein which functions by coordinating the assembly of a replication initiation complex at the viral replication origin [6]. Although E1 is mostly expressed in the upper basal layer of the skin and is not considered as an effective candidate HPV vaccine, several studies have demonstrated that E1 vaccination can either induce regression or provide protection against virus infection in different animal models [7–13]. We chose five naturally existing HLA-A2.1 restricted epitopes from CRPVE1 based on an online MHCI epitope prediction software program to design a multivalent epitope DNA vaccine [1].

Transgenic and non-transgenic rabbits from both outbred and EIII/JC inbred genetic backgrounds were used in our experiments. After one, two or three immunizations, the rabbits were challenged with wild type CRPV and a codon optimized CRPV DNA (manuscript in preparation) on left and right back sites respectively. Papilloma appearance and size were monitored weekly 3 weeks after DNA challenge. Our results showed that all HLA-A2.1 transgenic rabbits from both inbred and outbred genetic backgrounds were completely protected from CRPV challenge after two immunizations when compared to vaccinated control rabbits. An additional therapeutic study indicated that this multivalent E1 epitope DNA vaccine could trigger papilloma regression and size reduction in some rabbits. These data indicate that E1 can be a potential candidate for both preventive and therapeutic vaccines.

Materials and Methods

Peptide and tetramer synthesis

HLA-A2.1 restricted epitopes from CRPVE1 were identified using MHCI epitope prediction software and five epitopes (CRPVE1 42–50 SLLDDTDQV; 149–157 ILNANTARV; 161–169 LLFRQAHSV; 245–253 ALLSQLLGV and 303–311 MLQEKPFQL) were selected for construction of a multivalent epitope vaccine. The peptides were synthesized in the core facility of Penn State University College of Medicine and verified by mass spectroscopy.

DNA vaccine

The five selected A2 restricted epitopes from CRPVE1 were engineered into a DNA vaccine and separated one from another with alanine-alanine-tyrosine (AAY) spacers [14] as reported previously [15]. The construct also included a Kozak sequence at the N-terminus, followed by a tetanus toxoid (TT) universal T-helper motif (QYIKANSKFIGITEL) [16] and a Ubiquitin motif [17–19] (Ubiquitin A76 DNA, a kind gift from Dr. J. Lindsay Whitton, the Scripps Research Institute, La Jolla, CA) at the C-terminus. DNA-coding sequences were remodeled to remove potential internal translation initiation sequences and extended tandem or inverted repeats. When not countermanded by these restrictions, codon optimization was also employed in constructing this plasmid DNA vaccine. The synthetic nucleotide sequence was then incorporated into an expression vector (pCX) driven by a chicken beta-actin promoter. The final constructs were identified as CRPVE1ep1-5 and Ub3 respectively. The final concentration of the plasmids was adjusted to 1 µg/µl in 1× TE buffer and then precipitated onto 1.6 µm-diameter gold micro particles at a ratio of 1 µg of DNA/0.5 mg of gold particles as described by the manufacturer (Bio-Rad, Hercules, California) [20].

Peptide binding assay

The peptide binding experiments were conducted following the method reported previously with some modification [14]. T2 (a human HLA-A2.1 cell line) was used for the assay. In brief, naturally bound peptides were stripped from the HLA-A2.1 molecules by exposing the T2 cells for 90 seconds to ice-cold citric acid buffer, pH3.1 (1:1 mixture of 0.263 M citric acid and 0.123 M Na₂HPO₄). Cells were immediately buffered with ice-cold fetal bovine serum and washed twice in complete medium, and re-suspended in complete medium containing 2 µg/ml human β₂-microglobulin (Sigma-Aldrich). Subsequently, the stripped T2 cells were plated at 4×10⁴/well in a 96-well U-bottomed plates together with 20 µg/ml peptides. After incubation at 37°C for 4 hrs, cells were washed three times in PBS containing 2% FBS. The cells were then incubated with a specific anti-HLA-A2.1 monoclonal antibody (BB7.2, ATCC) and phycoerythrin (PE)-conjugated goat-anti-mouse IgG. After washing three times, the cells were fixed with PBS containing 2% paraformaldehyde, and analyzed on a FACScan™ flow cytometer (Becton Dickinson).

T2 cell-stabilization assay

A T2 cell-stabilization assay was also performed as described previously with some modification [21]. T2 cells were seeded into 96-well U-bottom plates at a density of 3×10⁵/ml and incubated for 18 h with each peptide (20 µg/ml) at 26 °C. Cultured cells in triplet wells were washed three times at 2, 4 and 6 hours later and stained with mouse anti-human HLA-A2 antibody (BB7.2) and subsequently with PE-conjugated goat anti-mouse IgG. Samples were analyzed on a FACScan™ flow cytometer (Becton Dickinson). Binding activity of each peptide was calculated by a fluorescence ratio (mean fluorescence of T2 cells loaded with peptide: mean fluorescence of T2 cells without peptide).

Rabbit vaccination and challenge

HLA-A2.1 transgenic and non-transgenic rabbits with both outbred and EIII/JC inbred genetic background were maintained in the animal facility of the Pennsylvania State University College of Medicine. All animal care and handling procedures were approved by the Institutional Animal Care and Use committee. Inner ear skin sites were shaved and swabbed with 70% ethanol, and then DNA/gold particles were bombarded onto these sites by a gene gun at 400 lb/in² [20]. Animals were immunized with CRPVE1ep1-5 or Ub3 for three times at 3-week intervals. Each vaccine DNA was applied at a total dose of 20 µg per rabbit for each immunization. Two weeks after the final booster immunization, rabbits were challenged with two progressive CRPV DNA at four left and right back sites respectively (10 µg construct/site):

wild type CRPV DNA (wtCRPV) which showed a progressive phenotype in previous studies [20] and a codon optimized CRPV DNA (coCRPV), with 15 and 14 codon optimized in E6 and E7 respectively, that showed a more progressive phenotype when compared to the wild type CRPV (Cladel, NM, unpublished observations)

For therapeutic vaccination experiments, rabbits were challenged with wtCRPV and a coCRPV DNA at four left and right back sites respectively (10 μ g construct/site). Eight weeks after infection, the rabbits were immunized with CRPVE1ep1-5 for three times at monthly intervals. Papilloma measurements began 3 weeks after DNA challenge and continued weekly for 12 weeks.

Papilloma size determination and statistical analysis

Papilloma size was determined by calculating the cubic root of the product of length $\times$ width $\times$ height of individual papillomas in millimeters to obtain a geometric mean diameter (GMD). Data were represented as the means ($\pm$ SEMs) of the GMDs for each test group. Statistical significance was determined by unpaired *t*-test comparison ($P < 0.05$ was considered significant). The percentage of sites without papillomas was calculated as the number of sites without papillomas/total number of challenged sites. Statistical significance was determined by the Fisher's Exact Test ($P < 0.05$ was considered significant).

Results

Prediction of HLA-A2.1 restricted epitopes with online software

CRPVE1 was screened for HLA-A2.1 epitopes using different MHCI epitope prediction software online and five HLA-A2.1 restricted epitopes were chosen based on their score (Table 1, <http://www.sbc.su.se/svmhc/new.cgi>).

Affinity of CRPVE1 HLA-A2.1 restricted epitopes

Corresponding peptides of five HLA-A2.1 restricted CRPVE1 epitopes were synthesized (20mg/ml in stock). T2 cells were pulsed with 20 μ g/ml of each individual peptide and stained for A2 surface expression. T2 cells pulsed with HPV16 E7/82–90 were used as a positive control, and T2 cells without peptide were used as a negative control. Mean fluorescence intensity (MFI) values were used to assess binding affinity. A ratio (MFI of tested peptide/medium control) of more than three was considered positive binding. All five chosen epitopes showed good binding compared to the positive control epitope (Fig 1A).

We next determined the stability of the HLA-A2.1/epitope complex. A T2-stabilization assay was used for this purpose. T2 cells cocultured with different peptides overnight. HLA-A2.1 expression levels were examined at 2, 4 and 6 hours after peptides washed off. Our results showed that all five peptides formed stable complexes with HLA-A2.1 six hours after removal of the unbound peptides. Significant differences in binding stability were found for these five E1 peptides when compared to medium or a negative control peptide (Fig 1B). Compare with other three peptides, NDCP1 and NDCP2 showed relatively weaker affinity to HLA-A2.1 but the peptide/HLA-A2.1 complex were just as stable (Fig 1B).

Three immunizations provided complete protection against viral DNA challenge in HLA-A2.1 transgenic rabbits

HLA-A2.1 transgenic and control outbred rabbits were immunized with E1 epitope vaccine and control vaccine three times ($N=4$) respectively at 3-week intervals. The animals were challenged with wild type CRPV DNA and a mutant progressive CRPV DNA on their left and right back sites respectively. All challenge sites on E1-vaccinated HLA-A2.1 transgenic rabbits were protected from both viral DNA infections (Table 2). There was one tiny papilloma on

one of the E1-immunized HLA-A2.1 rabbits which regressed two weeks later. In contrast, significantly more challenge sites on control-vaccinated rabbits grew papillomas (Table 2). The mean papilloma size was significantly larger in these control rabbits (Fig 2A, 2B $P < 0.01$, unpaired t test).

HLA-A2.1 transgenic and normal EIII/JC inbred rabbits were also immunized with E1 epitope vaccine and control vaccine three times ($N=3$) respectively at 3-week intervals. The animals were subsequently challenged with wtCRPV DNA and coCRPV DNA as described previously. All challenge sites on HLA-A2.1 transgenic EIII/JC inbred rabbits immunized with CRPVE1ep1-5 were protected from both viral DNA infections (Table 3). Interestingly, no challenge sites on Ub3 immunized HLA-A2.1 transgenic EIII/JC inbred rabbits grew papillomas from wtCRPV DNA challenge but 50% of sites challenged with coCRPV grew papillomas. Furthermore, CRPVE1ep1-5 provided partial protection against wtCRPV DNA and complete protection against coCRPV DNA in normal EIII/JC inbred rabbits. In contrast, almost all the sites challenged with both constructs grew papillomas in normal EIII/JC inbred rabbits immunized with Ub3 (Table 3). The mean papilloma size was significantly larger in these animals (group 4) when compared to those of group 1–3 (Table 3, Fig 3A–B, $P < 0.01$, unpaired student test). Because non-transgenic rabbits vaccinated with the CRPVE1ep1-5 showed higher protection rates against CRPV infection when compared with non-transgenic animals in Ub3 group ($P < 0.05$, the Fischer's exact test, Table 3), it may indicate that the E1 epitopes also activate immunity restricted to endogenous rabbit MHCI molecules.

Two immunizations were sufficient to provide complete protection against viral DNA challenge in HLA-A2.1 transgenic rabbits

HLA-A2.1 outbred ($N=6$) and EIII/JC inbred ($N=4$) transgenic rabbits were immunized with CRPVE1ep1-5 and Ub3 two times respectively at 3-week intervals. The animals were challenged with wtCRPV DNA and coCRPV DNA on their left and right back sites respectively. Consistent with previous findings, all HLA-A2.1 transgenic rabbits immunized with CRPVE1ep1-5 were protected from both viral DNA infections (Table 4). In contrast, significantly more challenge sites on CRPVE1ep1-5 vaccinated non-transgenic rabbits grew papillomas (Table 4). The mean papilloma size was significantly larger in Ub3 rabbits (Fig 2C–2D, Fig 3C–D, $P < 0.01$, unpaired student t test).

One immunization failed to provide protection against viral DNA challenge in HLA-A2.1 transgenic rabbits

Two immunizations were sufficient to provide complete protection against viral infection in HLA-A2.1 transgenic rabbits. We next tested whether one immunization could also provide protection. HLA-A2.1 transgenic and non-transgenic rabbits were immunized with CRPVE1ep1-5 DNA vaccine and Ub3 vaccine ($N=6$) respectively. The animals were challenged with wtCRPV DNA and coCRPV DNA on their left and right back sites respectively one week after the immunization. No protection was found in HLA-A2.1 transgenic rabbits immunized with CRPVE1ep1-5 (data not shown). The mean papilloma size induced by both wtCRPV and coCRPV was also comparable between HLA-A2.1 transgenic rabbits and non-transgenic rabbits (data not shown).

Therapeutic immunization triggered papilloma regression in both HLA-A2.1 and normal rabbits

Eight weeks after viral DNA challenge, HLA-A2.1 and normal rabbits were divided equally into two groups ($N=5$). These animals were then immunized with CRPVE1ep1-5 or Ub3 two times with 3-weeks intervals. The papillomas were monitored weekly before and after treatment. Two out of two HLA-A2.1 transgenic rabbits and two out of two normal EIII/JC inbred rabbits responded to CRPVE1ep1-5 treatment resulting in papilloma regression or

reduction in size (Table 5). No reduction in papilloma size was observed in rabbits from outbred and inbred genetic background vaccinated with Ub3. In addition, two outbred rabbits in this control group develop cancer after 6 months of infection (Table 5).

Discussion

We designed a multivalent epitope DNA vaccine by linking five HLA-A2.1 restricted CRPVE1 epitopes selected by online MHCI epitope predication software. This multivalent epitope DNA vaccine stimulated complete and specific protective immunity in the HLA-A2.1 transgenic rabbit model system. These data indicate that our HLA-A2.1 transgenic rabbit model can be used to test the immunogenicity of predicted epitopes by online software and be an excellent preclinical model for the development of effective prophylactic and therapeutic vaccines.

HLA-A2.1 transgenic rabbit model was established and reported previously [1]. Because CRPV DNA can be modified extensively without losing its ability to induce papillomas on animals, we are able to examine efficiency and specificity of different vaccine candidates in this model system [22]. Most of the CRPV early proteins are immunogenic and can provide partial or complete protection against viral infection by stimulating cell-mediated immune responses in rabbits [12,23]. In contrast, late proteins (L1 and L2) predominantly stimulate strong humoral immune responses that result in complete protection against virus infection [24,25]. More recently, we showed that L1 can stimulate cell-mediated immune responses in addition to neutralizing antibody [26]. Although virus-like particle vaccines have been successfully used clinically, no effective therapeutic vaccine has been reported so far [2]. VLP vaccines, which are excellent for stimulating protective immunity, may not necessarily work well for cancer treatment. Based on this collective information, we predicted that different epitopes from different genes might be targeted by host immunity at different levels and stages of disease.

Previous studies have shown that E1 is a good candidate for protective immunity in rabbits [8,12]. However, the precise epitopes and immune responses that are targeted by the rabbit immune system has not been determined. In this study, we screened HLA-A2.1 specific epitopes of CRPV E1 and generated a multivalent epitope DNA vaccine. We hypothesized that if these epitopes are the correct targets for activation of protective CD8 responses and do not cross react with rabbit MHCI, they should provide protective immunity in HLA-A2.1 transgenic rabbits but not in normal rabbits. Our data showed complete protection was achieved in HLA-A2.1 transgenic rabbits. Interestingly, increased protection in normal rabbits was also found especially in inbred rabbits that were immunized with this epitope vaccine. In addition, results from a therapeutic study showed papillomas on two HLA-A2.1 transgenic rabbits and normal inbred rabbits vaccinated with this epitope vaccine shrank or regressed. These data indicate that a potential cross reactivity of some of these epitopes may exist in which binding to rabbit MHCI might occur. To determine which of these outcomes are possible, we have initiated a study in which individual E1 epitope DNA vaccines were prepared and tested for protective immunity in transgenic and normal inbred rabbits.

Our data shown here demonstrated that our HLA-A2.1 transgenic model can be used to identify and test immunogenic targets from candidate epitopes selected by online MHCI epitope predication software. Based on our experiments with mutagenesis, we demonstrated that the CRPV genome had a high capacity for modification without compromising its ability to induce papillomas in rabbits [22]. This characteristic allows us to embed HLA-A2.1 epitopes from other human pathogens into CRPV genes. Combined with this epitope DNA vaccination strategy, we can provide *in vivo* data to test the efficacy of different candidate epitopes for both prophylactic and therapeutic vaccines.

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Figure 1.

A) T2 binding assay for the peptides. NDCP1-P5 represented CRPVE1/161–169, CRPVE1/245–253, CRPVE1/42–50, CRPVE1/303–311 and CRPVE1/149–157 respectively. NDCP6 (HPV16E7/82–90) and medium were positive and negative controls. T2 cells were pulsed with each peptide (10 μ M) and MFI of A2 expression were detected with flow cytometry. B). HLA-A2.1/peptide complex stabilization assay. NC34 (HTLV-1 Tax/67–75) and NC36 (HPV16E7/82–90) were used as controls. T2 cells were cultured with the peptide overnight and the peptide was removed. At 2, 4 and 6 hours after the peptide removal, T2 cells were labeled for A2 expression.

Figure 2.

Papilloma outgrowth in outbred HLA-A2.1 transgenic and control rabbits after viral DNA challenge. HLA-A2.1 transgenic and control outbred rabbits immunized with CRPVE1ep1-5 three (A, B) and two times (C, D) were challenged with wild type (A, C) and codon optimized (B, D) CRPV DNA. HLA-A2.1 transgenic rabbits were completely protected from both wild type and codon optimized CRPV DNA challenge in both experiments. Significantly smaller papillomas were found in all immunized A2 rabbits ($P < 0.01$, unpaired student t test).

Figure 3.

Papilloma outgrowth in EIII/JC inbred HLA-A2.1 transgenic and control rabbits after viral DNA challenge. HLA-A2.1 transgenic and control inbred rabbits immunized with CRPVE1ep1-5 three (A, B) and two times (C, D) were challenged with wild type (A, C) and codon optimized (B, D) CRPV DNA. HLA-A2.1 transgenic rabbits were completely protected from both wild type and codon optimized CRPV DNA challenge in both experiments. Significantly smaller papillomas were found in all immunized A2 rabbits ($P < 0.01$, unpaired student t test).

Table 1
Scores for HLA-A2.1 restricted CRPVE1 epitopes from different MHCI epitope prediction software

Antigen	Epitopes name	Position	Peptide Sequence	BIMAS Scores	SYFPEITHI Scores	MHCPEP Scores
CRPVE1	NDCP1	161–169	LLFRQAHSV	437,482	1.31	1.03
	NDCP2	245–253	ALLSQLLGV	591,888	1.19	0.99
	NDCP3	42–50	SLLDDTDQV	517,001	1.03	0.87
	NDCP4	303–311	MLQEKPFQL	863,596	0.69	1.29
	NDCP5	149–157	ILNANTARV	118,238	0.96	0.93

Papilloma appearance in NZW **outbred** HLA-A2.1 transgenic and normal rabbits challenged with wild type and codon optimized CRPV DNA after *three* immunizations with CRPVElep1-5 DNA vaccine

Table 2

Groups	Rabbits	Vaccine	Challenge Sites	Sites with Papillomas	
				Wild type CRPV DNA	Codon optimized CRPV DNA
1	A2 (N=4)	Elep1-5	16	0/16 (0%) ^{a,b,c}	0/16 (0%) ^{a,b,c}
2	A2 (N=4)	Ub3	16	13/16 (81%)	15/16 (94%)
3	Control (N=2)	Elep1-5	8	5/8 (63%)	7/8 (88%)
4	Control (N=3)	Ub3	12	10/12 (83%)	11/12 (92%)

^aP=0.001

^bP=0.011

^cP=0.002 vs. group 2, 3 and 4 respectively, Fisher's Exact Test

Table 3
Papilloma appearance in EIII/JC **inbred** HLA-A2.1 transgenic and normal rabbits challenged with wild type and codon optimized CRPV DNA after *three immunizations* with CRPVE1ep1-5 DNA vaccine

Groups	Rabbits	Vaccine	Challenge Sites	Sites with Papillomas	
				Wild type CRPV DNA	Codon optimized CRPV DNA
1	A2 (N=3)	E1ep1-5	12	0/12 (0%) ^a	0/12 (0%) ^d
2	A2 (N=3)	Ub3	12	0/12 (0%) ^b	6/12 (50%) ^e
3	Control (N=3)	E1ep1-5	12	3/12 (25%) ^c	0/12 (0%) ^f
4	Control (N=3)	Ub3	12	11/12 (92%)	12/12 (100%)

^aP=0.005

^bP=0.005

^cP=0.101

^dP=0.003

^eP=0.353

^fP=0.003 vs. group 4 respectively, Fisher's Exact Test

Papilloma appearance in NZW **outbred** and EIII/JC inbred HLA-A2.1 transgenic and normal rabbits challenged with wild type and codon optimized CRPV DNA after *two* immunizations with CRPVElep1-5 vaccine

Table 4

Groups	Rabbits	Genetic background	Vaccine	Challenge Sites	Sites with Papillomas		
					Wild type CRPV DNA	CRPV DNA	Codon optimized CRPV DNA
1	A2 (N=6)	Outbred	Elep1-5	24	0/24 (0%) ^a	0/24 (0%) ^b	0/24 (0%) ^b
2	Control (N=6)		Elep1-5	24	8/24 (33%)	12/24 (50%)	12/24 (50%)
3	A2 (N=5)	EIII/JC Inbred	Elep1-5	20	0/20 (0%) ^c	0/20 (0%) ^d	0/20 (0%) ^d
4	Control (N=4)		Elep1-5	16	8/16 (50%)	8/16 (50%)	8/16 (50%)

^a P=0.007

^b P=0.001 vs. control group 2

^{c,d} P=0.004 vs. control group 4, Fisher's Exact test

Table 5

Papilloma outgrowth after therapeutic immunizations with CRPVE1ep1-5 in HLA-A2.1 transgenic and normal rabbits

Rabbit ID	Vaccine	Mean Papilloma size (GMD in mm)	
		Before treatment	6 months after treatment
E0109(672) [*]	Ub3	14.05±1.54	12.35±2.37
E0111(674) [*]		7.50±0.59	9.75±0.69
E0106(665) ^{**}		8.31±2.45	Cancer
E0105(670) ^{**}		13.75±0.45	Cancer
E0163(684) [#]		11.70±1.30	11.56±1.32
E0155(676) [*]	E1ep1-5	14.74±1.42	0
E0162(683) [*]		9.81±0.57	1.79±1.06
E0165(690) [#]		9.34±1.10	0
E0166(691) [#]		11.23±1.12	4.99±1.08
E0103(668) ^{**}		10.05±1.07	11.59±0.171

^{*} HLA-A2.1 transgenic rabbits[#] Normal EIII/JC inbred rabbits^{**} Normal outbred rabbits