

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Kenter GG, Welters MJP, Valentijn ARPM, et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. *N Engl J Med* 2009;361:1838-47.

NEJM 08-10097 R4 (*web-only version*)

Description and Composition of the Medicinal Product

The medicinal product consists of a subcutaneous vaccine and contains 9 HPV16-E6 synthetic peptides and 4 HPV16-E7 synthetic peptides with the following sequences: E6₁₋₃₂: MHQKRTAMFQDPQERPRKLPQLCTELQTTIHD; E6₁₉₋₅₀: LPQLCTELQTTI HDIILECVYCKQQLLRREVY; E6₄₁₋₆₅: KQQLLRREVYDFAFRDLCIVYRDGN; E6₅₅₋₈₀: RDLCIVYRDGNPYAVCDKCLKFYSKI; E6₇₁₋₉₅: DKCLKFYSKISEYRHYCYSLYGTTL; E6₈₅₋₁₀₉: HYCYSLYGTTLQYQYNKPLCDLLIR; E6₉₁₋₁₂₀: YGTTLQYQYNKPLCDLLIRCINCQKPLCPEEK; E6₁₀₉₋₁₄₀: RCINCQKPLCPEEKQRHLDDKKQRFHNIRGRWT; E6₁₂₇₋₁₅₈: DKKQRFHNIRGRWTGRCMSSCRSSRTRRETQL; E7₁₋₃₅: MHGDTPTLHEYMLDLQPETTDLYCYEQLNDSSEEE; E7₂₂₋₅₆: LYCYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIVT; E7₄₃₋₇₇: GQAEPDRAHYNIVTFCKCDSTLRLCVQSTHVDIR; E7₆₄₋₉₈: TLRLCVQSTHVDIRTLEDLLMGTGIVCPICSQKP. The peptides are dissolved in DMSO / 20 mM PBS (pH 7.5) and emulsified with Montanide ISA-51 in a final DMSO/PBS/Montanide ratio of 20/30/50 v/v/v. One dose of each vaccine contains 0.3 mg of each peptide in a total volume of 2.8 ml.

Immunomonitoring of HPV16-specific T-cell responses

Peripheral blood mononuclear cells (PBMC) were isolated from fresh heparinized blood samples by Ficoll density gradient according to the manufacturer. The PBMC were directly used for lymphocyte stimulation tests and the remaining PBMC were cryopreserved. HPV16-

specific T-cell responses were determined by a previously described set of complementary assays, all described in standard operation procedure protocols and performed by trained personnel, using predefined criteria for positive and vaccine-induced responses.¹⁵ Proliferative capacity of the T cells in response to HPV16 E6 and E7 peptide stimulation was assessed by a 7-day lymphocyte stimulation test (LST) using freshly isolated PBMC. Supernatants obtained at day 6 were subjected to cytokine analysis by cytometric bead array (human Th1/Th2 CBA-kit, BD Pharmingen). Quantitative analysis of the number of circulating HPV16 E6- and/or E7-specific IFN γ -producing T cells was determined by a 4-day IFN γ -ELISPOT in which simultaneously cryopreserved PBMC isolated before and after vaccination were tested. Phenotyping of the HPV16-specific IFN γ -producing population was done by intracellular cytokine staining (ICS) with antibodies against CD4, CD8, and IFN γ . For the screening of CD4⁺ T-cell responses by LST, IFN γ -ELISPOT and ICS the PBMC were stimulated with a set of 22 amino acid long peptides, overlapping by 12 amino acids. The peptides were mixed into four pools of E6 peptides and two pools of E7 peptides resulting in a total of 6 different peptide pools used to stimulate the PBMC of each patient.¹⁵ For the analysis of CD8⁺ T-cell responses by ICS PBMC were stimulated with pools of 10 amino acid long overlapping peptides. Each peptide displayed an overlap of 9 amino acids and was used at 10 peptides per pool.¹⁵

Web Table Local adverse events according to CTCAE grading system in 22 patients who received at least 1 vaccination.

(- = none; +/- = slightly; + = mild; ++ = moderate; +++ = severe)

*: if a reaction occurred more than once in a patient, this patient was only counted once

** : maximum of any vaccination site at any time during the study

	Patients* (N=22)	%	Vaccinations (N=81)	%
Pain	21	95	57	70.4
-	1	4.5	24	29.6
+/-	6	27.3	31	38.3
+	12	54.5	21	25.9
++	0	0.0	2	2.5
+++	3	13.6	3	3.7
Redness	22	100	80	98
-	0	0.0	1	1.2
+/-	1	4.5	9	11.1
+	4	18.2	36	44.4
++	14	63.6	31	38.3
+++	3	13.6	4	4.9
Skin temperature higher	22	100	79	97.5
-	0	0.0	2	2.5
+/-	0	0.0	10	12.3
+	3	13.6	20	24.7
++	13	59.1	40	49.4
+++	6	27.3	9	11.1
Swelling**	22	100	81	100
2-4 cm	0	0	8	9
4-6 cm	3	13	30	37
6-8 cm	7	31.8	25	30.9
8-10 cm	11	50	17	21
>10 cm	1	4.5	1	1.2
Skin ulcer	1	4.5	1	1.2