

# Ex vivo monitoring of cellular memory responses in young women immunized with either Gardasil® or Cervarix™ four years prior to enrolment

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## BACKGROUND

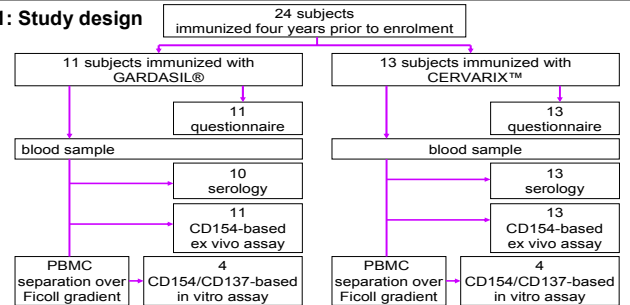
The virus-like-particle (VLP)-based quadrivalent AAHS-adjuvated vaccine Gardasil® and bivalent AS04-adjuvated vaccine Cervarix™ provide prophylactic protection against infections with the human papilloma virus types 6/11/16/18 and 16/18, respectively. Serological data from large phase III/III clinical trials showed sustained antibody titers, above those following natural infection. Cellular immune responses, in particular T helper cell responses, are important for both induction and maintenance of humoral responses.

To assess cellular immune responses to both vaccines we monitored HPV antigen-specific CD4 and CD8 T cells from whole blood and peripheral blood mononuclear cell (PBMC)-suspension using a CD154-based *ex vivo* as well as CD154/CD137-based *in vitro* assay.

## METHODS

We conducted a non-randomized, cross-sectional study including 24 subjects, immunized with either Gardasil® (11) or Cervarix™ (13) four years prior to enrolment (Fig. 1). Whole blood and PBMC-suspension were stimulated with different HPV-L1 and HPV-E6/E7 peptide pools for 14-20 hours or 10 days. PBMC-suspensions were restimulated on day 10 with respective peptide pools. Antigen specific memory CD4 and CD8 T cells were identified by intracellular staining for CD4/CD154/IL-2/IL-4/IFN $\gamma$  or CD8/CD137/IL-2/IFN $\gamma$  and analysed by flow cytometry (Fig. 2 and 3). Blood plasma was analysed for the presence of antibodies to HPV-proteins by multiplex HPV serology based on *in situ*-purified glutathione S-transferase fusion proteins.

Fig. 1: Study design



CD154-based ex vivo assay			
Direct access to CD4+ T cells specific for defined antigens according to CD154			
To our question adapted method			
STIMULATION	FIXATION	STAINING	
Blood sampling	Brefeldine A secretion block	EDTA treatment	Permeabilization
Stimulation with antigens:	Erythrocyte lysis	Intracellular staining	
Control	Formalin fixation	CD4	
6L1*		CD154	
11L1*		IL-2	
16L1*		IL-4	
18L1*		IFN $\gamma$	
16/18 E6/E7*			
6 E6/E7*			
TT			
SEB			
* HPV peptide pools			
0 h	2 h	14-20h	16-22 h

Fig. 2: CD154-based ex vivo assay

L= late protein; E=early protein; TT= tetanus toxoid; SEB= staphylococcal enterotoxin B; CD= cluster of differentiation; IL= interleukin; IFN $\gamma$ = interferone gamma.

CD154/CD137-based in vitro assay						
day 0	day 3	day 6	day 10	day 11		
PBMC separation over Ficoll gradient	50% fresh medium + IL-2/IL-7	50% fresh medium alone	RESTIMULATION with respective antigens	Brefeldine A secretion block	EDTA treatment	Permeabilization
PBMC in medium* + IL-2/IL-7					Formalin FIXATION	Intracellular STAINING
Control						CD4
6L1						CD154
11L1						IL-2
16L1						IFN $\gamma$
18L1						or CD8
16/18 E6/E7						CD137
6 E6/E7						IL-2
						IFN $\gamma$
						IFN $\gamma$
HPV peptide pools						
0 h	2 h	14-20h	16-22 h			

Fig. 3: CD154/CD137-based in vitro assay

L= late protein; E=early protein; CD= cluster of differentiation; IL= interleukin; IFN $\gamma$ = interferone gamma.

## RESULTS

Four years after vaccination with either Gardasil® or Cervarix™, vaccine-type specific memory CD4+ T cells are detected *ex vivo* in all vaccinees (Fig. 4). In comparison to month 7 CD4+ T cell frequencies at 4 years post vaccination decrease to comparable levels for both vaccines (please compare to O-10.03, P-13.15). Interestingly, women immunized with Cervarix™ show also CD4+ T cell response to low-risk HPV types. Antibodies to vaccine-type HPV are detected in all vaccinees. As expected antibody titers and CD4 T cell responses to low-risk types are higher in Gardasil vaccinees. HPV 16 antibody titers and CD4+ T cells are marginally higher in Cervarix™ vaccinated subjects (Fig. 5) whereas HPV18 responses are similar.

*In vitro* stimulation leads to a significant expansion of vaccine-type specific CD4+ T cells (Fig. 6). Vaccine-type specific CD8+ T cells are detected *in vitro* in most of the vaccinees at very low frequencies only after restimulation (data not shown).

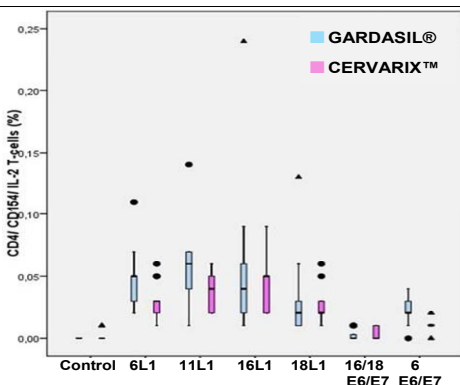


Fig. 4: CD4+ T cell response to *ex vivo* stimulation

CD= cluster of differentiation; IL= Interleukin; L= late protein; E= early protein.

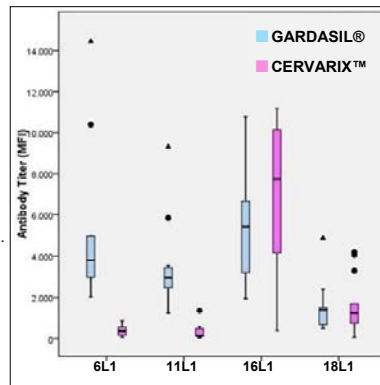


Fig. 5: Serology

MFI = median fluorescence intensity; L= late protein.

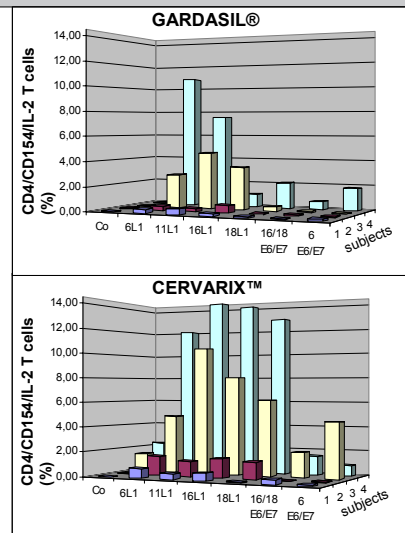


Fig. 6: CD4+ T cell response to *in vitro* stimulation

CD= cluster of differentiation; IL= Interleukin; Co= control; L= late protein; E= early protein.

## CONCLUSIONS

HPV vaccines induce long lasting memory CD4+ T cell responses that may support sustained antibody concentrations and may be important for boosting if necessary. Cellular immune response to low-risk HPV types in Cervarix™ vaccinees is possibly due to cross reactivity between various HPV types or previous infection with the respective HPV type.