

A prospective study on the natural course of low-grade squamous intraepithelial lesions and the presence of HPV16 E2-, E6- and E7-specific T-cell responses

Yin Ling Woo^{1,2}, Muriel van den Hende⁴, Jane C. Sterling¹, Nicholas Coleman³, Robin A.F. Crawford², Kitty M.C. Kwappenberg⁵, Margaret A. Stanley¹, Sjoerd H. van der Burg⁵

¹Department of Pathology, Cambridge University, Cambridge CB2 2QQ, United Kingdom

²Department of Gynaecology Oncology, Addenbrooke's Hospital, Cambridge CB2 2QQ, United Kingdom

³MRC Cancer Cell Unit, Hutchison/MRC Research Centre, Cambridge CB2 2XZ, United Kingdom

⁴Department of Gynaecology, Leiden University Medical Centre, Albinusdreef 2, 2300 RC Leiden, The Netherlands

⁵Department of Clinical Oncology, Leiden University Medical Centre, Albinusdreef 2, 2300 RC Leiden, The Netherlands

This study investigates the clinical course of low grade squamous intraepithelial lesions (LSIL), HPV status and HPV16-specific immune response in a large prospective study of 125 women with LSIL followed cytologically, virologically and histologically. Women with low-grade abnormal smears were recruited and followed-up for one year. Colposcopy, cervical biopsy for histology and brushings for HPV typing was performed at recruitment, 6 months (no biopsy) and upon completion of the study at one year. HPV16-specific T-cell responses were analysed by interferon- γ ELISPOT at entry, 6 and 12 months. Infection with multiple HPV types was detected in 70% of all patients, HPV16 was found in 42% of the patients. LSIL lesions progressed to HSIL in 24%, persisted in 60% and regressed to normal in 16% of the patients. No difference was observed in the clearance rate of infections with single or multiple HPV types among the groups with a different histological outcome. HPV16-specific type 1 T-cell responses were detected in only half of the patients with an HPV16+ LSIL, and predominantly reactive to HPV16 E2 and E6. Interestingly, the presence of HPV16 E2-specific T-cell responses correlated with absence of progression of HPV16+ lesions ($p = 0.005$) while the detection of HPV16 E6 specific reactivity was associated with persistence ($p = 0.05$). This large prospective study showed that the majority of LSIL persisted or progressed within the first year. This was paralleled by immune failure as most of the patients with an HPV16+ LSIL failed to react to peptides of HPV16 E2, E6 or E7.

Cervical cancer is preceded by a spectrum of epithelial atypia known as cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesions (SIL) characterized by disturbances of cellular maturation, stratification and cytological atypia

Key words: HPV, LSIL, CIN, immunotherapy, vaccines

Abbreviations: HPV: human papillomavirus; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial neoplasia; PBMC: peripheral blood mononuclear cells; IFN: interferon; ELISPOT: Enzyme linked immunospot; PHA: phytohaemagglutinin; NTP: nucleotide triphosphate YLW and MvdH contributed equally to this article.

Grant sponsors: Cancer Research UK and Evelyn Trust, Gordon Hamilton-Fairley CRUK Clinical Research Training Fellowship;

Grant sponsor: NWO; **Grant number:** Zon/Mw 917-56-311;

Grant sponsor: NWO; **Grant number:** Zon/Mw 920-03-188

DOI: 10.1002/ijc.24804

History: Received 2 Jun 2009; Accepted 16 Jul 2009; Online 30 Jul 2009

Correspondence to: Sjoerd H. van der Burg, Department of Clinical Oncology, Building 1, KI-P, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands, Fax: +31-71-5266760, E-mail: shvdburg@lumc.nl

of increasing severity. CIN/SIL are caused by persistent infection with one of a subset of genital human papillomaviruses (HPV), in particular HPV type 16. Natural history studies¹ show that most (90%) low grade cervical intraepithelial neoplasia (CIN 1) regress spontaneously and this is attributed to the development of HPV antigen specific cellular immune responses² but 30–40% of CIN3 progress to invasive cervical cancer³ and spontaneous regression is relatively uncommon.

The key role of the adaptive cellular immune system in protection against HPV-induced lesions is indicated by the high incidence of persistent HPV-infections and subsequent HPV-related malignancies in immunosuppressed individuals⁴ and the observation that only a small fraction of infected non-immunosuppressed subjects develop progressing epithelial lesions or cancer.⁵ Composite data indicate the importance of CD4+ T-cells in the control of HPV-induced diseases as more severe lesions are observed in patients with low numbers of circulating CD4+ cells^{6,7} and the increase in CD4 count, after anti-retroviral treatment, correlates with the regression of HPV-induced CIN lesions in HIV + patients.⁸ At the time of spontaneous regression of HPV infected genital warts, the lesions are infiltrated with CD8+ cytotoxic T-cells (CTL), CD4+ T-cells and macrophages.⁹ HPV16