HPV16-Specific T-Cell Responses and Spontaneous Regression of High-Grade Anal Intraepithelial Neoplasia

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Background

- High-grade squamous intraepithelial lesions (HSILs) are detected in ~33% of asymptomatic men who have sex with men (MSM). Less than 1% per year progress to cancer, therefore most lesions persist or spontaneously regress.
- SPANC (Study of the Prevention of Anal Cancer) is a prospective natural history study of anal SILs in HIV- and HIV+ MSM aged ≥35 years in Sydney, Australia, with 5 visits over 3 years.
- Our aim was to describe HPV16-specific T-cell responses in a cross-section of SPANC participants and correlate these with anal HSILs.
- We hypothesised that detectable HPV16-specific T-cell responses would be associated with recent anal HSIL regression.

Methods

- We used two assays for detecting HPV16-E6 and -E7-specific T-cell responses: 1) a novel assay for antigen-specific CD4+ T-cell responses based on CD25/CD134 co-expression; and 2) an intracellular cytokine staining (ICS) assay for antigen-specific CD4+ and CD8+ T-cells based on IFNγ and IL2 production.
- HPV DNA detection and genotyping was performed on contemporary liquid-based anal cytology swabs using the Roche (Alameda, CA) Linear Array test.
- Cross-sectional associations were tested for using Pearson χ^2 tests for dichotomous variables.

Results

Characteristics of the 134 SPANC Immunology Substudy participants are shown in **Table 1**, and results of T-cell assays are shown in **Table 2**.

Table 1 – Participant characteristics

	HIV-uninfected	HIV-infected	All
N (%)	103 (76.9)	31 (23.1)	134 (100)
Age [years, mean (SD)]	51.2 (9.58)	49.5 (8.33)	50.8 (9.31)
Anal cytology [N (%)]	103 (100)	31 (100)	134 (100)
Negative / Unsatisfactory	47 (45.6)	14 (45.2)	61 (45.6)
LSIL / ASC-US	22 (21.4)	8 (25.8)	30 (22.4)
HSIL / ASC-H	34 (33.0)	9 (25.8)	43 (32.1)
HRA [N (%)]	103 (100)	31 (100)	134 (100)
Visually normal (no biopsy)	27 (26.2)*	3 (9.7)*	30 (22.4)
Visually abnormal (≥ 1 biopsy)	76 (73.8)	28 (90.3)	104 (77.6)
Most serious anal histology [N (%)]	76 (100)	28 (100)	104 (100)
Negative	28 (36.8)	7 (25.0)	35 (33.7)
LSIL	16 (21.1)	6 (21.4)	22 (21.1)
HSIL (AIN2/3)	32 (42.1)	15 (53.6)	47 (45.2)
Anal HPV tested [N (%)]	99 (100)^	31 (100)	130 (100)^
Concurrent HPV16 detected	26 (26.3)	12 (38.7)	38 (29.2)
Any high-risk HPV type detected	55 (55.6)	22 (71.0)	77 (59.2)
Absolute T-cell count [cells/μL, mean (SD)]			
CD4+	822 (254)	584 (250)	766 (271)
CD8+	465 (198)	859 (496)	556 (337)

*P = 0.05. ^Four participants were unassessable on the HPV typing assay

Table 2 – T-cell assay results (N=134)

	CD4+ Positive responses [N (%)] *Median response size [% of parent population (IQR)]*		CD8+ Positive responses [N (%)] *Median response size [% of parent population (IQR)]*	
CD25/CD134 Assay				
Zero	0 (0) -		-	
SEB	134 (100) *9.520 (6.450 – 13.200)*		-	
CMV	131 (97.8) *2.980 (1.870 – 4.780)*		-	
HPV16-E6	68 (50.8) *0.062 (0.036 – 0.137)*		-	
HPV16-E7	32 (23.9) *0.075 (0.041 – 0.146)*		-	
ICS Assay	**IFNγ**	**IL2**	**IFNγ**	**IL2**
Zero	0 (0) -	0 (0) -	0 (0) -	0 (0) -
SEB	131 (97.8) *1.060 (0.509 – 2.110)*	132 (98.5) *2.210 (1.190 – 3.670)*	133 (99.3) *3.380 (1.550 – 5.870)*	126 (94.0) *1.035 (0.494 – 1.850)*
CMV / CEF	117 (87.3) *0.818 (0.390 – 1.800)*	110 (82.1) *0.231 (0.110 – 0.525)*	121 (90.3) *0.650 (0.230 – 1.430)*	80 (59.7) *0.160 (0.092 – 0.452)*
HPV16-E6	17 (12.7) *0.073 (0.052 – 0.120)*	9 (6.7) *0.049 (0.033 – 0.096)*	24 (17.9) *0.105 (0.074 – 0.143)*	5 (3.7) *0.208 (0.105 – 0.256)*
HPV16-E7	0 (0) -	16 (11.9) *0.075 (0.051 – 0.142)*	20 (14.9) *0.144 (0.086 – 0.300)*	6 (4.5) *0.118 (0.057 – 0.206)*

Abbreviations: SEB = staphylococcal enterotoxin B; CMV = cytomegalovirus lysate; CEF = CMV, EBV & influenza peptides.

Results (continued)

- HIV+ men were more likely to have CD8+ T-cell responses (IFNγ+ and/or IL2+) to HPV16-E6 (32.3%) than HIV-uninfected men (14.6%, P = 0.027), independent of absolute CD8+ T-cell counts.
- Men with high-grade disease (cytological HSIL and/or histological AIN2/3) were more likely to have CD8+ T-cell responses (IFNγ+ and/or IL2+) to HPV16-E7 compared to men without (29.4% vs.12.1%, P = 0.012).
- 26 men had high-grade disease at study entry a mean one year (SD 6.4 months) prior. Of these men, 6 (23%) experienced spontaneous regression by the time these assays were performed (see **Figure 1**). Five (83%) of these 6 regressors had CD4+ T-cell responses to HPV16-E6 on the CD25/CD134 assay, vs. 7 of 20 (35%) non-regressors (P = 0.037).

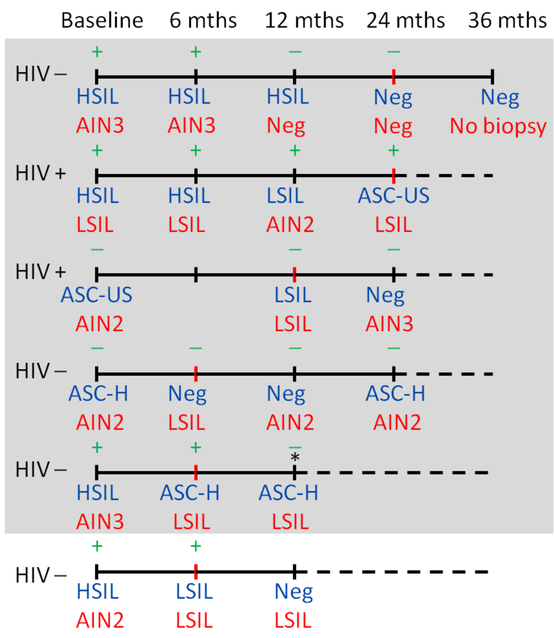


Figure 1 - Clinical course of the 6 participants with baseline high-grade disease who experienced spontaneous regression

*Incident HIV infection

Legend: HPV16 status Cytology diagnosis Histology diagnosis
Solid line = completed visits; Dashed line = future visits;
Red visit mark = visit at which T-cell assays were performed.
Grey background = participants with detectable HPV16-E6-specific CD4+ T-cell responses on CD25/CD134 assay.

Conclusions

- Systemic CD4+ T-cell responses were common in this cohort, with 2/3 of MSM positive to HPV16-E6 and 1/3 to HPV16-E7.
- Systemic CD8+ T-cell responses to HPV16-E6 or HPV16-E7 were detected in 20%.
- HIV+ men and men with high-grade disease were more likely to have CD8+ T-cell responses to HPV16-E6 and HPV16-E7, respectively.
- HPV16-E6-specific CD4+ T-cell responses were associated with recent anal HSIL spontaneous regression.
- The novel CD25/CD134 assay may have potential as an immune-based biomarker in screening algorithms for anal cancer prevention in MSM.

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