

SYSTEMIC T LYMPHOCYTE RESPONSES TO HPV 16 E6 AND E7 PEPTIDES AND LOCAL CERVICAL INFLAMMATORY CHANGES IN WOMEN WITH CIN III TREATED WITH HEAT SHOCK PROTEIN-BASED IMMUNOTHERAPY

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ABSTRACT

OBJECTIVES: SGN-00101 (HspE7, Nventa, San Diego, CA) is a novel therapeutic vaccine composed of a fusion protein with an M. Bovis BCG heat shock protein (Hsp65) covalently linked to the entire sequence of HPV 16 E7. We present some of the translational work of a Phase II trial (NCI 5850) that was designed to evaluate the efficacy and toxicities of HspE7 immunotherapy in HIV(-) women with CIN III. Specific aims were to examine: 1) The association between in-vitro systemic T cell responses to pooled HPV 16 E6 and E7 peptides and clinical response and 2) The relationship between clinical outcome and inflammatory changes in post-vaccination LEEP specimens.

METHODS: Women with biopsy-proven CIN III underwent three monthly subcutaneous vaccinations of 500 µg of HspE7 followed by an observation period of up to 7 months following by a LEEP. Venipuncture was performed at 5 of the clinical visits and PBMCs were cryopreserved. CD4 and CD8 positive T cells were activated with pooled E6 and E7 peptides and tested for interferon gamma production and degranulation (CD107) staining using flow cytometry. Blinded histologic sections of the final cervical conization specimens were independently graded for inflammation using a standardized protocol (0 no inflammation and 4 the highest level of inflammation). Standard clinical response evaluations were made based on the final cervical cone biopsy pathology.

RESULTS: A total of 58 evaluable patients completed the vaccinations and underwent cervical conization. There was significantly more stromal inflammation in the cervix specimens from patients who did not undergo regression (p=0.04 using Mann-Whitney test). Inflammation did not correlate with type-specific HPV infection. Preliminary data reveal no correlation between clinical response to vaccination and in vitro T cell responses.

CONCLUSIONS: There was significantly more cervical inflammation in patients who did not undergo regression after HspE7 immunotherapy. Local inflammation did not correlate with type-specific HPV infection or systemic T cell responses to HPV peptides; however, this study may be underpowered to predict these observations in this subset of women with heterogeneous T cell responses. Further studies to examine the T-cell subsets that are locally and systemically correlated with clinical regression to immunotherapy are currently underway.

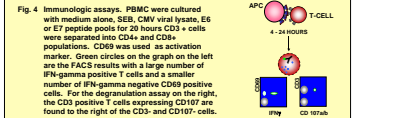
METHODS

Local inflammation:

All LEEP specimens underwent blinded grading for lesional and non-lesional inflammation (0-4+) as well as thorough documentation of the presence of megacell, koilocytosis, cervicitis, and squamous metaplasia by the study pathologists. Associations between inflammation and clinical response were correlated using the Wilcoxon rank sum test.

Immune responses:

- Cryopreserved serum and PBMC's from multiple visits
- Examine T-cell responses to pooled HPV 16 E6 and E7 peptides and normal recall antigens using multiple assays (Fig.4):
 - IFN using Cytokine Flow Cytometry
 - Degranulation assay (CD107 A and B)
- Correlate CMI responses to clinical status



INTRODUCTION

The inability of Human Papillomavirus (HPV)-infected women to generate effective HPV-specific T and B cell responses and eliminate HPV-infected cells contributes to the pathogenesis of CIN and invasive cancer. Current treatment strategies are aimed at destroying visible lesions. However, these ablative techniques are not effective in eradicating HPV from the genital tract.

The development of immunotherapeutic strategies for the management of anogenital cancer and associated HPV infection is progressing rapidly. There have been several recent reviews of progress in cervical cancer vaccine development⁵. Nevertheless, the discovery of appropriate reliable surrogate biomarkers that correlate with clinical response to therapy is not advancing in parallel. Current 'gold standard' HPV testing and histopathology provides some, but not all of the necessary information to adequately assess a patient's risk for persistence or progression of disease. Identification of 'protective' epitopes in the HPV E7 protein will facilitate the development of innovative immunotherapies for management of women with premalignant and malignant HPV-associated neoplasia.

Nventa Biopharmaceuticals Corp. (San Diego, CA) has produced an immunotherapeutic vaccine called SGN-00101 (HspE7) that consists of the M. Bovis BCG heat shock protein (Hsp65), covalently linked at its C terminus to the entire sequence of the HPV 16 E7 protein (Fig. 1). Heat shock proteins (Hsp) loaded with antigen elicit significant T and B cell responses against microbial pathogens and tumor antigens⁵⁻⁸ which are assumed to be partly responsible for clinical regression in vaccinated subjects. Clinical responses to HspE7 immunotherapy have been observed in children with recurrent respiratory papillomatosis⁹ and men and women with genital warts¹⁰ and anal intraepithelial neoplasia (AIN)¹¹. Since responses to HspE7 were seen in these other HPV-related diseases, we tested HspE7 in women with CIN III. The overall objective of the translational correlates for NCI 5850 is to determine whether patients who undergo regression after immunotherapy with SGN-00101 (HspE7) correlates with local immune responses and E7-specific proliferative and cytokine secreting memory T cell responses. In order to achieve these objectives, we performed the following specific aims:

- Identify local inflammatory responses and correlate them to clinical response
- Identify T-cell responses systemically to specific HPV peptides before and after vaccination with HspE7



Fig. 1 HspE7 (SGN-00101, Nventa Biopharmaceuticals, San Diego, CA- formerly Strenggen Biotechnologies). Construct consists of the M. bovis BCG heat shock protein (Hsp65), covalently linked at its C terminus to the entire sequence of the HPV 16 E7 protein

Brief Summary of results of NCI 5850 (for details see poster B203):

Adult women with colposcopically-directed biopsies showing histopathologic changes consistent with CIN III (CIN II-III or CIN III) meeting eligibility criteria were vaccinated with a series of 3 subcutaneous thigh injections of HspE7 (500 µg) followed by an observation period and LEEP as shown in Fig. 2:

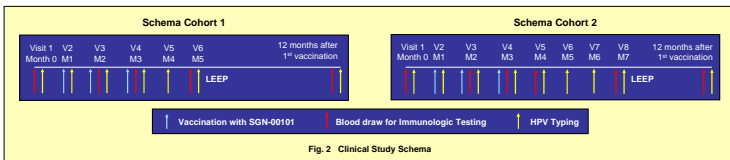


Fig. 2 Clinical Study Schema

- A clinical response was observed in 44/57 patients (77%, 95% CI = 66-88%)
- 12 (21%) had a complete pathologic response- absence of CIN (n=8) or CIN I (n=4) on final pathology
 - 32 (56%) had a partial response
 - 11 (19%) had stable disease
 - 2 (4%) had progressive disease (microinvasive SCC)
- No significant demographic, behavioral, or clinical risk factors associated with regression
- HspE7 was well tolerated in this patient population with local cutaneous rash (Fig. 3) being the most common side effect

Most common adverse events were related to injection site:

- Exanthem
- Pain
- Pruritis
- Swelling
- Shows local cutaneous immune response

Fig. 3 Side effects of SGN-00101 (HspE7)

RESULTS

- Upon thorough review of pathology by study pathologists, there appeared to be significant inflammation in post-treated LEEP specimens (Fig. 5). There was more inflammation in patients who did not undergo regression (p=0.04 using Mann-Whitney, Fig. 6). This may suggest a local T-suppressor immune response.
- Preliminarily, the T-cell responses to E6 and E7 peptides are very low (Table 1):
 - Women with CIS exhibit normal responses to controls (CMV and SEB)
 - Compared to women without CIN, E6 and E7 responses are very low in women with CIS prior to vaccination¹²
 - Immunocompetent patients with CIS of the cervix may have selective immunosuppression to HPV

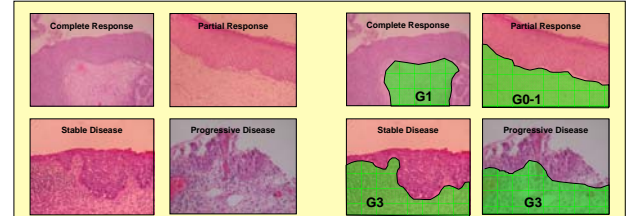


Fig. 5 Inflammation in LEEP specimens (100X Mag)

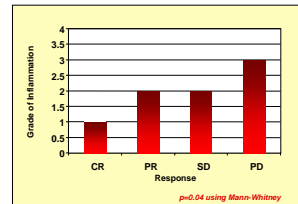


Fig. 6 Association between inflammation and response

Table 1: Immune response results to assays pre- and post-vaccination with HspE7

Degranulation Assay (CD107 a and b)					IFN-gamma Production						
Antigen	T-cell type	Number of subjects tested	Pre-Vac Median %T-cell response	Post-Vac Median %T-cell response	P Value (Mann-Whitney)	Antigen	T-cell type	Number of subjects tested	Pre-Vac Median %T-cell response	Post-Vac Median %T-cell response	P Value (Mann-Whitney)
E6 Peptide Pool	CD8	8	0.00	0.01	0.44	E6 Peptide Pool	CD8	8	0.00	0.00	0.79
	CD4	9	0.06	0.09	0.51		CD8	8	0.00	0.00	0.18
E7 Peptide Pool	CD8	9	0.00	0.06	0.10	E7 Peptide Pool	CD8	9	0.01	0.00	0.17
	CD4	7	0.07	0.02	0.37		CD4	9	0.00	0.00	0.96
CMV	CD8	9	0.00	0.00	0.79	CMV	CD8	8	0.15	0.03	0.27
	CD4	8	0.00	0.00	0.56		CD4	8	0.07	0.09	0.34
SEB	CD8	9	0.41	0.31	0.79	SEB	CD8	9	1.12	1.08	0.69
	CD4	8	0.13	0.12	0.34		CD4	8	0.945	1.36	0.56

FUTURE DIRECTIONS

- Evaluate the role of specific T-cell subsets in regression or persistence of CIN
- Look at T-suppressor responses in PBMCs (CD25+, FoxP3)
- Expanding our observations in women with normal Pap tests as a control
- Examine HPV L1 antibody responses and correlate to clinical regression
- Stain sections of LEEP specimens for specific T-cell subsets

CONCLUSIONS

- 1) Local inflammation correlated with regression after vaccination to HspE7. However, the inflammatory response is opposite what is hypothesized, i.e., patients with more inflammation were less likely to undergo regression. This suggests a T-suppressor response.
- 2) There is no correlation between immune responses to multiple assays and clinical response to vaccination with HspE7. However, this may be due to being underpowered to predict a difference in the multiple response subsets of this trial.
- 3) Immunocompetent patients (not anergic) with CIS of the cervix may have selective immunosuppression to HPV peptides.

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