DETECTION OF HIGH-RISK HPV-E6 ONCOPROTEIN IN LOW AND HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN): EMERGING EVIDENCE FOR PREDICTIVE VALIDITY

INTRODUCTION

High-risk HPV types cause over 95% of cervical cancers. Expression of high-risk HPV oncoproteins E6 and E7 is necessary for cervical epithelial cell transformation, cancer progression and maintenance. Therefore, E6 oncoprotein is potentially a diagnostic marker with predictive validity (Figure 4, schematic).

E6 of all high-risk HPV-E6 is to PDZ is the basis of a novel, cervical cancer diagnostic rapid immunoband. In HPV negative cervical swab material. Based on the concept of high-risk HPV-E6 as PDZs are conserved protein domains with widespread biological functions that include cell-to-cell contact, intercellular signaling, and cell polarity.

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RESULTS

Correlation of E6 expression with severity of cervical lesions.

Cervical swab specimen collected from women screened, diagnosed, and managed in rural settings in China were tested for the presence of HPV-16 DNA then examined for HPV16-E6 expression. E6 expression was found in 6 out of 7 CIN3 specimens, in 1 out of 2 CIN2 specimens, and in 1 out of 9 CIN1 specimens. Clients diagnosed with histologically confirmed CIN1 at baseline were enrolled in a cohort study where they were examined at one and two years. Year one follow-up included liquid-based cytology, HPV DNA testing via Hybrid Capture 2, and reflex colposcopy, biopsy, and ECC. Year two year, follow-up included liquid-based cytology, HPV DNA testing via Hybrid Capture 2, colposcopy, random and directed biopsy, and ECC. Most interestingly, the client with the E6 expressing CIN1 specimen progressed to CIN3 within one year with follow up examination via random biopsy. No progression to CIN3 was seen for baseline CIN1 clients whose specimens did not express E6 (Figure 3 and table in Figure 4). Comparison to E6 levels in SiHa cervical cancer cell lines. Limit of E6 detection is < 31000 cells (right).

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CONCLUSIONS

- E6 is a potential diagnostic marker with predictive validity. Abal number of samples are small, the first studies performed on cervical swab specimen collected from women in rural areas in China indicate a correlation between E6 expression and severity of disease (CIN1, 2 or 3) as well as the risk to develop severe lesions. These preliminary data are consistent with our current understanding of the natural history of cervical neoplasia and support the hypothesis that E6 is a diagnostic marker with predictive validity.

- E6 based diagnostic test for cervical pre-cancer and cancer.

Arbor Vita Corporation and PATH have developed a protocol of an E6 oncoprotein based novel rapid diagnostic assay for cervical pre-cancer and cancer. All high-risk HPV-E6 are specifically captured by a PDZ domain protein; detection occurs via anti E6 mAb. The assay format is lateral flow ("strip test"). Current sensitivity is ~ 150,000 SiHa cells.

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- The START project is supported by PATH through a grant from the Bill & Melinda Gates Foundation
- NIH SBIR 1 R43 CA123353-01 "A Novel Diagnostic for Oncoproteic HPV"
- NIH SBIR 1 R43 CA068190-01 "Rapid Strip Test for Cervical Cancer via HPV-E6 Detection"

Collaborators and consultants:
Arbor Vita Corp. maintains a collaboration with Dr. G. Ornthudt, G. Trowe and E. Weiss of the University Louis Pasteur of Strasbourg. One of the anti E6 mAbs ("4E2") used for this work was kindly provided by Dr. W. Weiss (Soinane C., et al., J. Minit. Racing 1999/12:141-152).

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PDZ Based High-Risk HPV-E6 Detection

Arbor Vita’s E6 based cervical cancer diagnostic: specific capture of high-risk HPV-E6 via a PDZ domain and detection via anti E6 mAbs (left).

Applying the sandwich ELISA principle shown left, E6 is detected in cervical cancer cell lines. Limit of E6 detection is ~ 31000 cells (right).

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E6 Levels Correlate with Severity of Lesions

Patients with CIN1 pathology are followed up for further disease progression; 2 of 3 CIN3 swabs were "secondary" collection swabs.

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E6 Based Lateral Flow CxCa Diagnostics

- Specific capture of high-risk HPV-E6 via a PDZ domain and detection via anti E6 mAb applied to lateral flow assay format (top left).

- Detection of HPV16-E6 from cervical cancer cells (SiHa) lines in the background of a HPV negative cervical swab specimen (top right).

- Workflow illustration of a cervical cancer rapid diagnostic assay (bottom).

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