Point of Care Cervical Cancer Screening for Worldwide Developing Markets

Technology Report

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Cancer is a complex and multifaceted disease that is caused by multiple genetic and biochemical abnormalities at a cellular level. Thus, the only method to fully characterize the cellular changes required for carcinogenesis, and to define the state of the disease, is to evaluate multiple biomarkers that reflect critical molecular changes associated with cancerous cells. OncoGenesis' paradigm is to quantify novel panel of biomarkers that are proven to be important in characterizing cervical cancers, and to quantify these biomarkers in relatively non-invasive cervical cytology samples using cutting-edge molecular detection technology platforms. Introduction of such system will fulfill important clinical unmet needs in the detection of abnormal gynecological lesions and will significantly reduce the mortality rate due to cervical cancers.

There is a growing awareness of the need for personalized, precise, biomarker-based approaches for all cancer diagnosis and treatment. Diagnostic companies have focused on lung, breast, colon and prostate cancers with market acceptance. Gynecologic cancers, especially the cervical cancer, remain an area of huge unmet need worldwide. OncoGenesis' biomarker-based cancer assays address this unmet need by providing reliable information that enables the gynecologic physician to identify patients with abnormal lesions that need to be treated before the disease progresses into cancer. The demand for personalized, cost efficient approaches to diagnose disease stage and determine the appropriate therapy drives physician and patient acceptance of molecular based diagnostics as a part of standard cancer care now and in the immediate future. OncoGenesis is developing novel biomarker test for cervical cancer that will be used for diagnosis, prognosis, and theranosis (tests that will guide therapy decisions) of cervical cancer.

CERVICAL CANCER BACKGROUND

Cervical Cancer is a disease characterized by abnormal or uncontrolled cell growth of cervical cells. It originates in the epithelial cells lining the interior and exterior surfaces of the cervix (i.e., "transformation zone", the point of entry into the uterus). It affects almost 500,000 women each year, with the highest incidence and death rates (about 85%) occurring in developing regions of the world where women have very limited access to routine gynecological exams [1,2].

The current process for cervical cancer screening, the Pap test, whether from a direct smear on a slide or a processed liquid specimen, relies on microscopic observation of abnormal epithelial cells identified in collected cervical specimens. The cytological processing is done in a laboratory and involves the analysis of the Pap smear under a microscope by highly trained cytologists (who are not readily available in developing

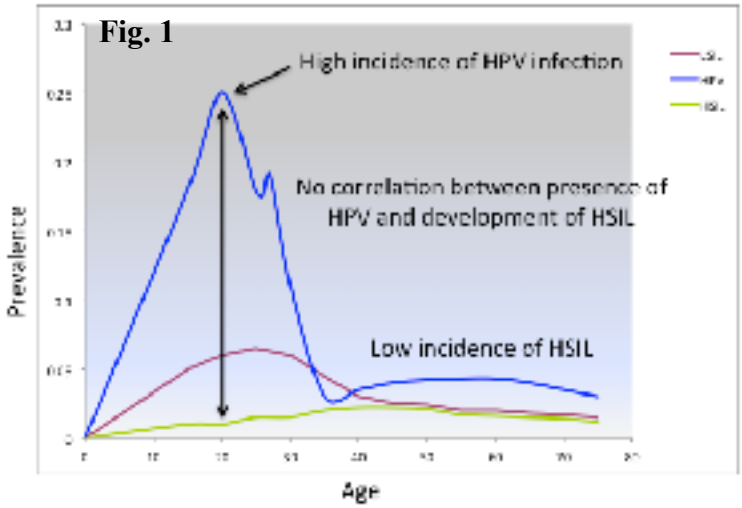
countries). The analysis is very subjective and results in 8% to 30% false positives and 8% to 30% false negatives, (depending on country) [4,5,6,7]. If you considered that 160 million Pap smears are completed annually worldwide, then at least 16 million false positives and at least 16 million false negatives.

A major finding relating to the etiology of cervical cancer is that it generally develops from a persistent infection caused by a high-risk type HPV, which infects epithelial cells. Fortunately, the majority of cases of HPV infection are cleared by the women's own immune system; however, in a small percentage (~10%) the virus integrates into the patient's genome, leading to measurable changes in specific protein production in cervical cells, and the development of dysplasia and neoplasia. It is for this reason that the American College of Obstetrics and Gynecology recently recommended a reduction in the number of screening tests in young women; it also highlights the problems with Pap and HPV test specificity [3,8].

An HPV vaccine is currently provided to girls and young women in developed nations with the intent of eliminating HPV infections, and thereby ultimately reducing the number of deaths from cervical cancer. It is important to note that this practice is not a replacement for cervical screening; the FDA requires continued routine screening for cervical cancer even after vaccination since the vaccine is not 100% effective. Furthermore, its adoption in many resource-limited areas are experiencing some compliance issues due to the inconvenience of sequential doses over extended periods of time and high cost of the vaccine [10,11,12].

In developing markets (e.g., India, China, Africa, etc.), a need for accurate molecular tests for cervical cancer screening that does not required trained personnel and expensive infrastructures is even greater [13]. Viable cervical screening test in the developing markets must involve an inexpensive, quick, disposable, molecular, cervical cancer screening system that is employed as a primary screen. Limited laboratory infrastructure and instrumentation should be required to quantitatively screen the cervical samples and provide analysis in minutes without the need for highly trained personnel and expensive infrastructure [14].

Recently, a FDA Advisory committee unanimously recommended HPV Testing as primary screening tool for detection of women at high risk for cervical cancer. Although this announcement highlights a need for more objective and accurate molecular tests to replace the Pap test, it also raises significant problems with using HPV tests as a primary cervical cancer-screening tool. The major problem with HPV test is that the HPV infection does not correlate



with abnormal high-grade lesions (HSIL) as shown in Fig. 1. Furthermore, although over 80% of women in the US will have acquired HPV infection by the age of 50, the vast majority of these women will be free of HPV infection without any treatment. Testing high-risk type of HPV, such as types 16 and 18, increase the probability of a patient's risk of developing a high-grade cervical lesion. Importantly, however the presence of this infection does not automatically means that the patient has a cervical lesion that requires therapeutic intervention. Moreover, although the presence of high-risk type HPV in samples, helps to identify patients at increased risk for development of disease, these analytes do not comment on the presence or current state of cervical lesions since HPV infection can be a cause but not necessary always the cause of cervical cancer. The only HPV tests marketed today require expensive laboratory infrastructure/personnel and ONLY determine if a virus is present – **they DO NOT detect or comment on the presence or the state of cervical lesions, and thus, makes the treatment decisions for patients with positive HPV test extremely difficult** [9].

ONCOGENESIS PARADIGM

Oncogenesis paradigm challenges the following traditionally accepted views on new medical devices:

1. *What works in U.S. and other developed countries will work in other countries.*

The medical devices and tests that were successfully employed and utilized in developed countries, such as U.S., may not be desirable or practical in countries with developing economies. Pap test and HPV testing are good examples of such tests. Although Pap test has been successful in reducing the number of cervical cancer, implementing the Pap test widely in developing countries is impractical since the developing countries lack the trained personnel and infrastructure to collect, process, and evaluate samples and to train new personnel. HPV testing is useful "adjunct" test to Pap test, but HPV tests are often expensive, and more critically, they are not diagnostic of the disease but are rather a "risk" indicator. Oncogenesis approach recognizes that the needs in developing countries are often very different from that of developed countries with more established medical infrastructure. Thus, Oncogenesis tests based on multiplex protein biomarkers that will provide objective diagnosis of the patients without a need for trained medical personnel is a solution to the problem whose time has come.

2. *Advanced technology is not appropriate for the needs of developing countries.*

There is a misconception that the needs in developing countries are simpler in nature so that the solution their problems do not require advanced technology. This type of thinking may be true for some instances, but is not true in detection and treatment of gynecological cancer, specifically cervical cancers. The solution to meeting the challenges associated with cervical cancer detecting in resource-limited countries can only be addressed through application of advanced technologies. Oncogenesis device

and test are based on the most advanced technologies currently available in medical field. Application and implementation of these technologies in the detection of cervical cancer will make significant impact in reducing the number of cervical cancer in developing countries.

3. *Developing countries cannot afford cutting-edge technologies in medical devices.*

Although some technologies in medical devices are indeed too expensive for a wide application in developing countries, the recent advances in material science and engineering allowed significant reduction in cost of development and manufacturing of advanced microfluidic device and biosensor. Oncogenesis devices are based on well-characterized designs that allow significant reduction in the cost of the instrument while still maintaining the engineering requirements. These factors allow development and wide implementation of objective, efficient, and cost-effective cervical cancer system a reality even in the developing countries.

CERVICAL CANCER BIOMARKERS

Cancer is a complex and multifaceted disease that requires multiple genetic and biochemical abnormalities at a cellular level. These abnormalities include growth deregulations, increased invasive properties, and prevention of programmed cell death. Thus, the only practical method to fully characterize key cellular changes during carcinogenesis is to use multiple biomarkers that will measure all of the critical cellular changes in a clinical sample. OncoGenesis' paradigm is to examine multiple biomarkers that are proven to be important in cervical cancer, and to measure the levels of these biomarkers in relatively non-invasive cervical cytology samples using cutting-edge molecular detection technology platforms**.** Evidence to support OncoGenesis' proposed paradigm and technological platform for cervical cancer screening are provided below. This includes peer-reviewed articles from various scientific or medical journals as well as summaries of data collected internally or through contracted efforts with outside academic and commercial partners.

OncoGenesis' choice of target biomarkers for specific and sensitive characterization of cervical cancer grade derives in part from work completed in Europe (Table 1). This work involved immunohistological and genomic testing of 302 biopsy samples against a panel of 13 different possible biomarkers **[15,16].** Analyses completed in this studies strongly demonstrates that a panel of 3 to 5 markers can be used to confidently grade cervical intraepithelial lesions. Furthermore, the data also suggest that high sensitivity and specificity for the detection of abnormal cervical lesions can be achieved by combining the markers in multiplex fashion. Additionally, peer-reviewed publications have been identified to provide evidence for the use of chosen targets as proven biomarkers for cervical cancer [17-23].

TABLE 1. Immunohistological Support for OncoGenesis Targeted Biomarkers

Marker	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Area under ROC curve (AUC)
			BIOPSY SERIES	n = 302	
Biomarker A	54.4	93.1	98.6	18.4	0.737
(Tumor suppressor, involvec in proliferation)					
Biomarker B	82.5	75.9	96.6	32.8	0.792
(Apoptosisinhibitor, involved in acoptosis)					
Biomarker C	88.0	80.0	97.4	40.0	0.800
(Extracellular matrix protein involved ir cell adhesion)					
Biomarker D	70.1	98.7	99.4	27.6	0.834
(Signalling proten, involved in intracellular signaling					
Biomarker A + B	85.7	73.9	96.7	36.1	0.798
Biomarker A + B + C	95.2	60.0	95.6	58.1	0.776
Biomarker A + B + D	91.3	73.8	96.6	47.8	0.823
			Hypothetical SCREEN	n = 10,000	
Biomarker A	54.4	99.3	44.7	99.5	0.772
Biomarker D	82.5	97.6	25.4	99.8	0.900
Biomarker C	88.0	97.9	29.2	99.9	0.919
Biomarker D	70.1	99.6	66.7	99.7	0.848
Biomarker A + B	85.7	97.4	24.5	99.9	0.917
Biomarker A + B + C	95.2	60.0	95.6	58.1	0.776
Biomarker A + B + D	91.3	73.8	96.6	47.8	0.823

Biopsy series: n=302; CIN1 (n=30, includes 10 cases of NCIN); CIN2 (n =21); CIN3 (n=101); SCC (n=150)

As a corollary to the cancer biomarkers used to determine the grade of cervical cancer, multiple publications support the detection of human papillomavirus as an important metric for cervical cancer screening. In cytological settings this may often involve genomic screening of patient samples for evidence of HPV nucleic acids. Published evidence of the value of HPV in cervical screening, as well as evidence supporting its direct link to elevation in target cervical cell biomarkers has been identified [24-27]. In a recent study by Yang et al. [35], they showed that measurement of HPV E6 protein in cervical cytology samples resulted in elevation of E6 protein expression in high-grade cervical cytology samples from histologically normal samples. This data supports our strategy that measurement of protein biomarkers in cervical cytology samples is a valid approach that can provide clinically useful information in identifying high-grade cervical lesions. OncoGenesis' platform will simultaneously detect evidence of HPV oncoprotein in the same patient sample evaluated for specific cervical cancer biomarker proteins.

<u>OncoGenesis Data</u>

Current efforts support the approach of using selected target proteins as an indicator of cervical lesions, detecting them in either whole cell or solubilized preparations. Results from several ELISA Immunodetection assays developed at OncoGenesis confirm the

utility of biomarkers; demonstrating successful detection within both cultured cervical cell lines or cervical cytology specimens.

Using the optimal sample preparation method in ELISA immunoassays, OncoGenesis tested levels of 3 biomarkers with a cellular internal control in an initial set of more than 200 cervical cytology specimens. The two studies shown conducted using remnant cervical cytology specimens collected at local and national reference laboratories. The samples were stored at either 4°C or room temperature for an average time of 43.4 days (range: 30-60) after collection by a health care professional. Samples were processed by a technique developed in our laboratory in which remnant preservative is removed and cells are concentrated prior to being lysed. This concentrated cell lysate was then used for quantification of specific cervical dysplasia biomarkers using our in-house immunoassay. The initial data show that the levels of all three biomarkers are elevated in high-grade cytology specimens when compared to normal controls. Even with limited number of samples, two of the three biomarkers achieved statistical significance in the separation of abnormal cervical lesions from normal controls.

These data are significant for the following reasons:

1. The data show that using the optimized sample preparation/extraction method, OncoGenesis biomarkers can be reliably recovered from the standard cervical cytology samples for testing.
2. The levels of biomarkers can differentiate samples from patients with abnormal high-grade cervical lesions from the normal controls.
3. These positive results were obtained even though we were using “old” specimens due to sample availability (stored 3-6 wks at room temperature prior to testing), indicating that the analytes are relatively stable in the ThinPrep liquid cytology preservative solution, and that the performance of our biomarkers could be increased with better quality samples.
4. Performance of the biomarkers obtained from histological samples observed in the previous studies could be reproduced in cervical cytology samples.

Table 2. Biomarkers that Defines Cancer

Biomarker	Details	Target(s)
Biomarker 1	Tumor suppressor	Indicates uncontrolled growth
Biomarker 2	Apoptosis inhibitor	Reflects prevention of cell death
Biomarker 3	Viral oncoproteins	Indicate oncogenic viral infection
Biomarker 4	Proliferation Marker	Implicates abnormal growth
Biomarker 5	Stem cell/reserve cell marker	Indicates more aggressive tumors
Biomarker 6	Housekeeping Protein	Normalization of cell sampling

The current panel of OncoGenesis biomarkers consists of biomarkers that are indicative of all critical molecular changes associated with cervical cancer progression (Table 2). Application of these biomarkers in cervical cytology samples will provide a comprehensive assessment of the state of cervical cells from the patients and will be able to identify patients with high-grade lesions who require immediate follow-up or treatment accurately and efficiently. This test will be particularly useful for triaging HPV positive patients who truly need follow-up and/or treatment from the vast majority of HPV positive individuals who do not have abnormal lesions and who do not need expensive and stressful follow-up procedures.

We are currently conducting a large multicenter study to examine the application of OncoGenesis biomarkers in cervical cancer specimens. This study combines samples from premier research institutions in the USA (e.g., UCLA, Stoneybrook, etc.) and samples from other countries such as Mexico and India.

PLATFORM & SENSORS

OncoGenesis cervical screening products provide an integrated approach to cervical screening that improves all aspects of delivery from collection through sample processing and analysis. Cervical samples collected via either OncoGenesis collectors or other clinical devices, are deposited in OncoGenesis collection vial, which provides important preprocessing steps prior to analysis on the OncoGenesis instrument platforms. OncoGenesis is developing two instrument platforms to introduce CerMark™ cervical cancer biomarker test. The two instrument platforms are:

1. **Oncogenesis Lab System**: Based on the existing multiplex detection systems through a partner, the Lab System is a laboratory instrumentation that will have higher sample testing capability and will be introduced to clinical laboratories.

2. **Oncogenesis POC system:** Designed as a novel stand-alone, point-of-care system for multiplex detection, the platform utilizes novel microfluidic architecture, providing full sample processing and testing capabilities amenable to low resource, remote locations.

Oncogenesis Lab system based on existing multiplex platform, will be introduced with an instrument partner for clinical labs. The launch of the Lab system will provide early validation of CerMark test in detecting abnormal cervical lesions in traditional liquid cytology samples. Even in developing countries, a significant number of cervical cytology samples are being analyzed in large clinical labs. Introduction of the Lab system will enhance earlier acceptance of the CerMark test while filling the needs of clinical laboratories where high-throughput analyses of samples is critical. We have evaluated several options for the appropriate multiplex instrument platform for the laboratory-based system. The final instrument platform for the Lab system will be provided in partnership with the instrument manufacturer and will require only minor

modifications and/or development effort to integrate CerMark test in a clinical laboratory setting.

Whereas the Oncogenesis Lab System platform will be marketed to typical laboratories associated with testing of cervical specimens, the Oncogenesis POC platform is leveraged for the launch of OncoGenesis point of care product. Instead of relying on traditional optical sensors however, the platform detects target antigens using revolutionary electrochemical sensor based unique interdigitated electrode arrays (IDEAs) for redox amplification [37]. Major advantage of the IDEA electrochemical biosensor over the typical optical detection method is that the electrochemical biosensor offers simpler measurements that can operate in turbid solutions that provide significant advantages in the analysis of biological samples. Furthermore, the IDEA sensor can be manufactured inexpensively using UV photolithograpy and can be miniaturized to fit any detection format [36]. This mode of detection provides improved stability and robust functionality demanded of point of care systems while providing high sensitivity.

Oncogenesis POC device will be designed to work effectively in developing countries where trained personnel or an access to clinical laboratory equipment are not readily available. Utilizing recent advances in microfluidic designs, OncoGenesis POC is designed to provide maximum biological information of the cervical cytology specimen with a minimal operator effort.

In general,

1. Cells collected and stored in preservative solution are separated from the preservative solution that interferes with the immunoassay.
2. The cells are then lysed to release protein extract that contains the relevant protein targets (biomarkers).
3. The biomarkers contained in the protein extracts are then detected using standard immunoassay (ELISA) with electrochemical detection.

Signals from each biomarker or control will be detected by an individual biosensor inserted into each detection chamber. Due to the simplicity and manufacturability of the IDEA biosensor, the cost of the biosensors will be minimal. By having the disposable biosensors embedded into the test cartridge, the complexity, size, and the cost of the instrument to run the test cartridge is greatly reduced.

CERVICAL COLLECTOR

iPap™ : Cervical Specimen Personal Collector (to be used by a woman to collect her own sample)

Although the development of sensitive, efficient, and cost-effective cervical cancer testing platform is important, the wide availability of cervical sample collection is critical element in reducing the rate of cervical cancer worldwide. Certain women are discouraged from going for regular cervical cancer screening due to cultural or religious reasons. For this reason, OncoGenesis is offering a simple, inexpensive, and convenient self-collection device that respects the individual privacy of women who might otherwise be reluctant to undergo screening. This device is designed to collect cervical cytology sample by the patients themselves without a need for a visit to the clinic. The kit is provided with a simple instruction, self-collector, and a vial of preservative to preserve the sample during transport once the samples are collected. These kits can be made available at local clinics or drug stores, where the women can use the kit to collect the samples by themselves and either mail the samples or drop them off at the local testing sites.

A preliminary feasibility study with 15 women has been conducted to determine the feasibility of adequate sample collection using OncoGenesis non-diagnostic personal collector. During the course of this study, participants also provided valuable feedback on the quality of the Instructions for Use document, along with suggestions for refinement of the design of the personal collector. The result of the study was promising that resulted in important design changes for the final design.

OncoGenesis is collaborating with Action Africa Help International in 2014 to conduct a funded study to investigate how a sample self-collection program (using iPap personal collector) should be implemented in Nairobi, Kenya. Representatives from government and health organizations will select and implement sample self-collection options for both urban and rural settings, i.e., preferred options that are logistically feasible, affordable and socially acceptable. The results of this study are anticipated to encourage government leaders of other countries and global organizations (e.g., WHO, PATH, Gates Foundation, etc.) to support the implementation of sample self-collection programs.

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