Optical Technologies and Molecular Imaging for Cervical Neoplasia: A Program Project Update

Timon P.H. Buys, PhD[1]; Scott B. Cantor, PhD[2]; Martial Guillaud, PhD[1]; Karen Adler-Storthz, PhD[3]; Dennis D. Cox, PhD[4]; Clement Okolo, MD[5]; Oyedunni Arulogon, PhD[6]; Oladimeji Oladepo, PhD[6]; Karen Basen-Engquist, PhD[7]; Eileen Shinn, PhD[7]; José-Miguel Yamal, PhD[8]; J. Robert Beck, MD[9]; Michael E. Scheurer, PhD[10]; Dirk van Niekerk, MD[11]; Anais Malpica, MD[12]; Jasenka Matisic, MD[11]; Gregg Staerkel, MD[12]; Edward Neely Atkinson, PhD[2]; Luc Bidaut, PhD[13]; Pierre Lane, PhD[1]; J. Lou Benedet, MD[14,15]; Dianne Miller, MD[14,15]; Tom Ehlen, MD, PhD[14,15]; Roderick Price, MSc[16]; Isaac F. Adewole, MD[17]; Calum MacAulay, PhD[1]; and Michele Follen, MD, PhD[16,18,19,*]

[1]Imaging Unit, Department of Integrative Oncology, British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada; [2]Department of Biostatistics, M.D. Anderson Cancer Center, University of Texas, Houston, Texas; [3]Department of Diagnostic Sciences, Dental Branch, Health Sciences Center, School of Public Health, University of Texas, Houston, Texas; [4]Department of Statistics, Rice University, Houston, Texas; [5]Department of Pathology, University of Ibadan College of Medicine, Ibadan, Nigeria; [6]Department of Promotion and Education, University of Ibadan College of Medicine, Ibadan, Nigeria; [7]Department of Behavioral Science, M.D. Anderson Cancer Center, University of Texas, Houston, Texas; [8]Division of Biostatistics, Health Sciences Center, School of Public Health, University of Texas, Houston, Texas; [9]Department of Population Science, Fox Chase Cancer Center, Philadelphia, Pennsylvania; [10]Department of Pediatrics and Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, Texas; [11]Department of Pathology, British Columbia Cancer Agency, Vancouver, British Columbia, Canada; [12]Department of Pathology, M.D. Anderson Cancer Center, University of Texas, Houston, Texas; [13]Department of Imaging Physics, M.D. Anderson Cancer Center, University of Texas Houston, Texas; [14]Department of Obstetrics and Gynaecology; University of British Columbia, Vancouver, British Columbia, Canada; [15]Department of Gynecologic Oncology, British Columbia Cancer Agency, Vancouver, British Columbia, Canada; [16]Department of Obstetrics and Gynecology, Drexel University, Philadelphia, Pennsylvania; [17]Department of Obstetrics and Gynaecology, University of Ibadan College of Medicine, Ibadan, Nigeria; [18]Department of Gynecologic Oncology, M.D. Anderson Cancer Center, University of Texas, Houston, Texas; and [19]Department of Obstetrics, Gynecology, and Reproductive Sciences, Health Sciences Center, University of Texas, Houston, Texas

ABSTRACT

There is an urgent global need for effective and affordable approaches to cervical cancer screening and diagnosis. In developing nations, cervical malignancies remain the leading cause of cancer-related deaths in women. This reality may be difficult to accept given that these deaths are largely preventable; where cervical screening programs have been implemented, cervical cancer–related deaths have decreased dramatically. In developed countries, the challenges of cervical disease stem from high costs and overtreatment. The National Cancer Institute–funded Program Project is evaluating the applicability of optical technologies in cervical cancer. The mandate of the project is to create tools for disease detection and diagnosis that are inexpensive, require minimal expertise, are more accurate than

*Currently, Department of Obstetrics and Gynecology, Drexel University, Philadelphia, Pennsylvania.

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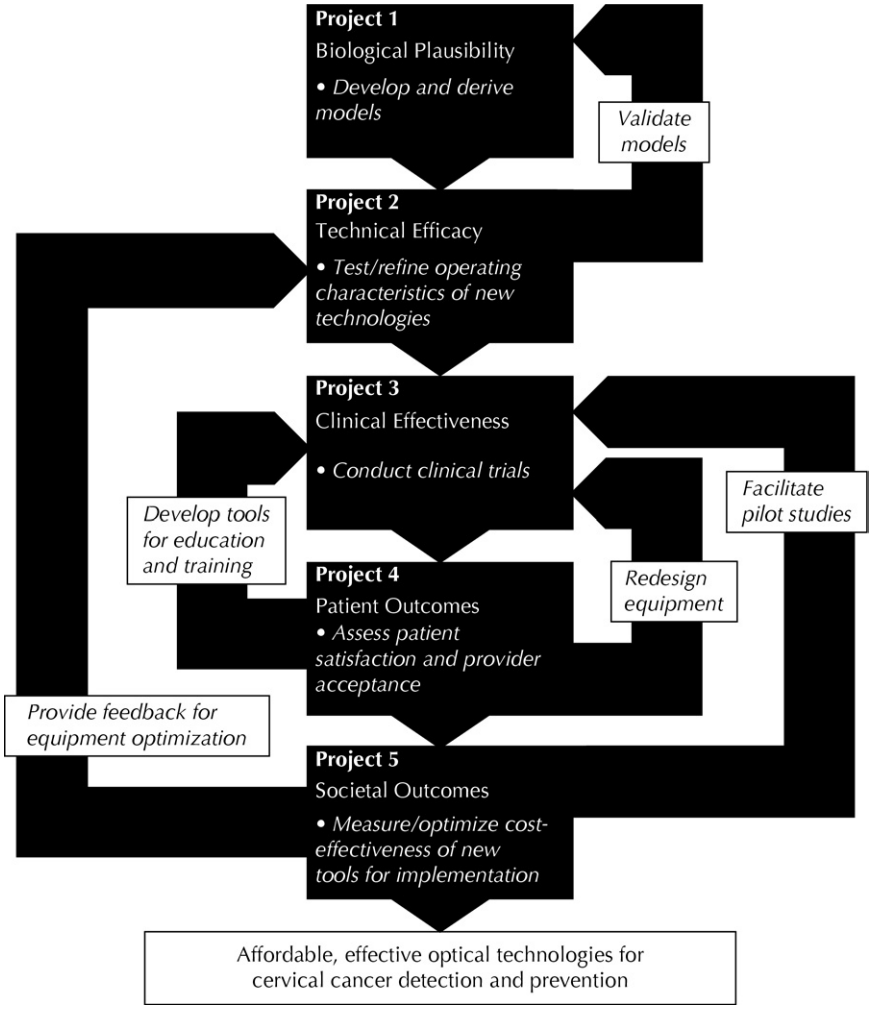

Figure 1. Framework of interaction between the 5 subprojects of the larger program project undertaking. Regular, effective interactions between project personnel have ensured that these connections remain robust.

existing modalities, and can be feasibly implemented in a variety of clinical settings. This article presents the status and long-term goals of the project. (*Gend Med.* 2012;9:S7–S24)

Key words: cervical cancer, HPV, human papillomavirus, screening.

INTRODUCTION

Eleven years ago, National Cancer Institute (NCI) funding facilitated assembly of a multidisciplinary research group to evaluate optical technologies for use in cervical cancer screening and diagnosis in both developed and developing nations. This team comprises optical engineers, gynecologic oncologists, clinician-pathologists, statisticians, computer scientists, epidemiologists, behavioral scientists, instrumentation engineers, decision scientists. This team launched an NCI-funded Program Project (P01 CA82710) of the biologic plausibility, technical efficacy, clinical effectiveness, patients' satisfaction, providers' satisfaction, and cost-effectiveness of optical technologies used for the detection of cervical cancer, as per the Littenberg technology assessment model[1] (**Figure 1**). Since the status of the project was last published,[2] it has progressed significantly. In addition, research on screening for cervical cancer has evolved. These changes—and the fact that cervical malignancies remain a leading cause of cancer-related deaths in women worldwide—make timely this updated summary of this translational work.

BACKGROUND

Cervical cancer remains the most common cause of cancer-related deaths in women in developing countries. In 2007, the number of newly diagnosed cases of cervical cancer was estimated to be 555,000 worldwide each year, with 310,000 cervical cancer–related deaths per year.[3] These rates of advanced-disease diagnoses and deaths are due in large part to the absence of effective prevention and screening programs. Where such programs have been implemented, the rates of invasive disease and cancer-related deaths have been reduced dramatically. For example, in British Columbia, Canada, where such a program has been implemented, the incidence of cervical cancer has declined by more than two thirds over the past 50 years.[4] Eighty percent of cervical cancer–related deaths occur in developing countries, where access to effective screening and treatments is limited.[3]

Approaches to Disease Screening and Prevention

There are several methods of preventing and detecting cervical neoplasias. The feasibility of adopting these methods vary by geographic region. The advent of vaccines against human papillomavirus (HPV) has the potential to greatly reduce the incidence of cervical cancer because this virus is a key etiologic factor in cervical neoplasia. HPV vaccines from Merck & Co., Inc. (Whitehouse Station, New Jersey) (covering HPV types 6, 11, 16, and 18) and GlaxoSmithKline (Brentford, United Kingdom) (covering HPV 16 and 18) are licensed for use in 60 countries.[5] At this time, these vaccines are administered only in developed countries, despite that those countries have the lowest rates of cervical cancer–related deaths worldwide (1 per 50,000 cases).[6–11] There are multiple challenges associated with administering HPV vaccines in developing countries. First, the cost is prohibitive; both the bivalent and quadrivalent commercially available HPV vaccines cost more than US $120/dose, and 3 doses are needed for the vaccines to be effective.[5] Second, it is challenging to achieve repeated clinical visits in the populations of developing countries; therefore, all 3 required doses may not be administered. Third, the continuous cold chain (ie, refrigeration) needed for vaccination materials is not feasible in some developing countries. Fourth, the HPV types highly prevalent in developing countries are not necessarily covered by the available vaccines.

Screening for HPV is being used in several clinical contexts, with polymerase chain reaction (PCR) analysis and the HPV Hybrid Capture II (HC2) test used for the detection of HPV DNA. These 2 assays can be used in several ways, including (1) for assessing cytologically defined low-grade squamous intraepithelial lesions (LSILs) and atypical squamous cells of uncertain significance (ASCUSs); (2) for follow-up of colposcopically negative but cytologically positive cases; (3) for predicting therapeutic outcomes; and (4) for a primary screening tool in the place of the Papanicolaou smear test. A meta-analysis addressed the use of HC2 and PCR in these 4 contexts and reported that no published studies had adequately addressed the utility of these approaches in colposcopically negative and cytologically positive cases.[12] In that same meta-analysis, great variability was reported in the specificity and sensitivity values calculated in each of the 4 contexts. For example, the specificities of PCR/HC2 in predicting treatment outcomes were reported to vary from 44% to 100%, depending on the study. Although the HC2 test is being studied in developed and developing countries, its $80 cost is prohibitive where resources are limited.[12–16] The careHPV test, developed by the Gates Foundation and QiagenCares (Valencia, California), was designed to be a cost-effective HPV DNA assay for developing countries. A clinical trial in China reported the sensitivity of careHPV to be 90% and the specificity, 85%, in predicting high-grade lesions (in patients evaluated only if 1 screening test was positive).[17] The potential utility of careHPV cannot be evaluated until its per-patient cost is known and a large-scale trial with histopathologic testing (for "gold-standard" comparison) has been completed.

Visual inspection with acetic acid (VIA) is a simpler screening methodology. It involves the application of acetic acid to the cervix, followed by visual examination of tissue with the naked eye and under white-light illumination for aceto-whit-

ening.[18,19] VIA is used in developing countries due its low cost, which results from the use of inexpensive reagents and minimal equipment and the ability to "see-and-treat" patients (precluding sample-handling expenses).[20] Although the sensitivity of VIA has been reported to be comparable to that of the Papanicolaou smear, its specificity been reported as lower.[21] Furthermore, aceto-whitening is not detected in ~20% of lesions detected using more robust methodologies.[21] More effective approaches are needed to detect cervical cancer in resource-poor settings.

An ideal approach to screening and diagnosis of cervical disease would have many specific traits. It would provide real-time results, obviating cumbersome follow-up appointments and procedures. Ideally, the same approach could be used in screening for cervical abnormalities in the general population and in a diagnostic setting (to properly classify disease stage). This ideal approach would also be inexpensive, thus facilitating its adoption in the developing nations where the majority of cervical cancer–related deaths occur. Its minimal training requirements would make wide and rapid dissemination of this approach possible. Sensitivity and specificity metrics for this approach would outperform those of established technologies.[20]

The researchers involved in the NCI project have spent the past decade evaluating the capacity of optical technologies to address these criteria in the context of cervical cancer.

Biological Plausibility

Dysplastic cervical tissues harbor many molecular alterations that govern cellular metabolic processes and change cell structures. Because various molecules found in tissue are natural fluorophores, the fluorescence of precancerous tissues differs from the fluorescence of normal tissues. Fluorescence spectroscopy represents a noninvasive approach for detecting tissues with such changes. For example, if collagen breaks down during the development of dysplasia and collagen fluoresces, then decreased fluorescence intensity in cervical tissues may be associated with the presence of diseased tissue. Testing has indeed demonstrated that this can occur in cervical tissues.[22] Multiple groups have reported that the use of techniques based on quantitative optical spectroscopy can improve early detection of cervical neoplasia, providing accurate, objective, and real-time diagnostic and screening tools.[23,24] The connection between these optical signatures and the underlying morphology and biology of diseased tissues is not completely understood.

Part of the reason for this incomplete understanding is that cervical tissues are complex and comprise many components. To diagnose disease, discrete measurements of individual tissue components (eg, nicotinamide adenine dinucleotide and its reduced form [NAD/NADH], flavin adenine dinucleotide and its reduced form [FAD/$FADH_2$], aromatic amino acid fluorescence at ultraviolet [UV] wavelengths) contribute to the understanding of the disease state. Excitation-emission matrices plot fluorescence intensities as a function of excitation and emission wavelengths, providing information about the behavior of many molecular components in tissue. Previously published studies have reported that fluorescence and reflectance spectroscopy can provide a highly informative readout for delineating healthy and premalignant tissues.[25–29]

The larger research project reported here has generated methods for quantifying cell morphology changes and yielded insights into changes to the molecular composition of cells during premalignant progression. For example, the research team developed electromagnetic computational tools that account for changes in nuclear structure while calculating the scattering of normal and dysplastic epithelial cells.[30] The research team also developed tools to measure scattering in normal and dysplastic epithelial cells from intact cervical tissues.[31] To verify the predictions provided by computer models, the research team used these tools to assess changes in scattering as a response to acetic acid and to help understand the effect of these changes on tissue spectroscopy.[32] The methods they are developing to measure the 3-dimensional distribution of nuclei will affect the emerging quantitative pathology tools being used elsewhere in the project.

To better calculate the scattering coefficient of structural protein networks, the research team enhanced existing electromagnetic computational tools, providing a means of evaluating how dysplastic changes lead to altered stromal scattering.[33] Going forward, these tools will facilitate quantitative studies of epithelial/stromal interactions. Noninvasive approaches based on these tools are being derived.

During the Program Project, the research team developed tools that measure fluorescence and reflectance to determine the optimal excitation wavelengths and source-detector separations for differentiating between neoplastic and non-neoplastic cervical tissue. Mathematical models of cervical tissue fluorescence and reflectance were then derived to understand these detected biophysical differences in vivo. Monte Carlo simulations were undertaken to validate these models, which were then used to analyze in vivo spectra accumulated during large screening and diagnostic trials associated with this project.[34,35] Based on these reflectance and fluorescence data and the use of these model parameters, diagnostic algorithms were derived with performance similar to empiric data-decomposition methods (eg, principal component analysis). An advantage of the model is that it may also be analyzed to extract information regarding the biological basis of disease (as reflected in the results from fluorescence and reflectance spectroscopy).[23,25,27,36–40]

Additional tools and models developed by the research team has contributed to the ability to detect and define the biological changes that underlie cervical dysplasia. To evaluate the fluorescence and reflectance spectra of tissue from different depths within the epithelium and stroma, the research team developed a fiber optic probe capable of selectively recording optical signals from the epithelium versus stroma. This device may yield enhanced diagnostic capability, particularly as it may be able to discriminate columnar tissues from dysplastic ones.[41] The research team's developed automated multispectral imaging approaches for the detection of cervical dysplasia. Models of fluorescence were used to select optimal wavelengths and collection geometries for imaging systems.[42–46] Upcoming pilot trials will test these metrics. An inverse model capable of distinguishing high-grade lesions and cancers from other cervical tissues also contributes to the research team's best-performing algorithm for delineating cervical lesions.[47] They are working to model cervical carcinogenesis and nuclear architecture using confocal microscopy to gain further insight into the disease.[30,48,49] With this work with both forward and inverse models, the biological basis for fluorophore changes observed in the histopathologic sections taken in the clinical trial setting can be better understood.

Technical Feasibility

In the current standard of care, patients in whom cervical abnormalities are detected using the Papanicolaou smear are referred for colposcopic examination. Diagnoses are then made based on histopathologic examination of colposcopically directed biopsies. This process may take days or weeks to complete and may require additional clinic visits for patients. It can add financial costs and anxiety in health care providers and patients. During the present Program Project, the research team has worked to facilitate real-time diagnosis of cervical abnormalities with novel imaging devices and techniques for quantitative fluorescence and reflectance spectroscopy. Efficacy of these techniques would help to improve standard approaches to the identification and treatment of cervical disease. More specifically, the research team's ongoing efforts involve the evaluation of several existing and emerging approaches for the detection and diagnosis of cervical disease: colposcopy, repeat Papanicolaou smear, endocervical curettage, point probe optical spectroscopy, multispectral digital colposcopy (MDC), VIA, and HPV testing. The research team is also evaluating various combinations of these approaches and devising analytical techniques for the improvement of the diagnostic performance of generated data. This work has been undertaken in the United States, Canada, and Nigeria, generating meaningful data from varied populations. Based on the findings from a literature search, these represent the largest trials of optical spectroscopy with statistically

justified sample sizes and consensus-read biopsies taken at each cervical site for use in gold-standard comparisons.

To test efficacy of each device, thousands of measurements were elicited from patients. A key lesson after evaluating 1850 patients and 4767 biopsies was that instrument noise can be a significant barrier to disease detection. One challenge of this project has been identifying sources random or systematic noise produced when measurements were obtained. Whenever possible, the research team sought to eliminate this noise using laboratory experiments and pilot trials. By having biostatisticians and engineers working in concert to address this challenge, better technology performance and stronger study design were facilitated. Given the challenges associated with evaluating device algorithms (although the trials were finished ahead of schedule, the selection of a best-performing algorithm for analysis took >3 years), it was extremely beneficial to have these diverse researchers in regular contact.

The research team has done extensive work in developing and optimizing a point probe for fluorescence and reflectance spectroscopy that measures a 2 × 2-mm area of the cervix (**Figure 2**). Some of the initial assessments of performance for this device were undertaken in vitro or on ex vivo tissues.[31,50,51] In early in vivo studies, baseline measurements for this device were made from normal epithelial, dysplastic, and tumor tissues.[25,26,31] The research team also evaluated the influence of several variables on device measurements, including the influence of point probe pressure on the cervix and the impact of having different devices and device users.[40,52–56] The impact of the menstrual cycle on fluorescence spectroscopy measurements was also examined.[57–59] Daily replicated measurements in women with no history of abnormal Papanicolaou smears suggested that intra-individual variation over the course of the menstrual cycle did not significantly affect measurements (although analysis during menstrual bleeding should be avoided, because it is likely the cause of observed variation).

The research team also sought to determine which device readouts provided the best diagnostic performance, deriving algorithms for analyzing fluorescence and reflectance spectra that were capable of delineating normal tissues, low-grade lesions, high-grade lesions, and tumor tissues.[23,60] Analytical approaches further evaluated spatial changes in spectra and biological insights into disease (eg, Monte Carlo modeling[34,35,39]). The research team found that the point probe device accurately delineated high-grade squamous intraepithelial lesions (HSILs) from all other types of epithelium—normal and abnormal, squamous and columnar—with a sensitivity of 80% and a specificity of 60% to 70%. Additional work on instrumentation and data algorithms has

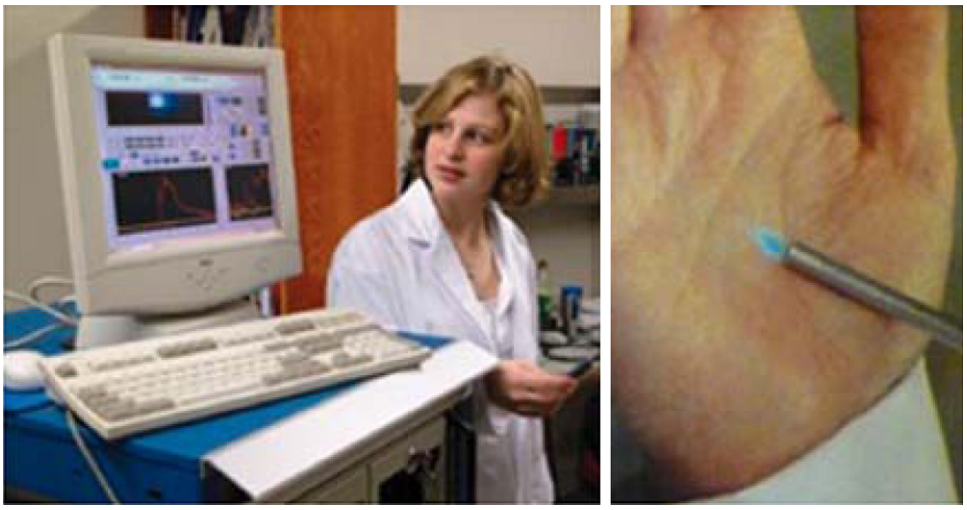

Figure 2. The point probe device. Computer equipment and readouts are shown (left), as is the point probe itself (right).

Figure 3. The multispectral digital colposcope (MDC). The left panel shows the imaging component of the MDC; the right panel shows the associated computer equipment required to operate the device and store imaging data.

improved these values, with sensitivity measured at 100% and specificity at 71%.[61] These values suggest that the point probe device has equivalent or better performance than colposcopy in the diagnostic setting. This in turn suggests that the point probe could be used as the first effective adjunct to colposcopy. The research team is currently working to further improve the detection capabilities of this device, allowing it to function independently from colposcopy.

Another major product of this project has been the development and optimization of MDC (**Figure 3**). This device visualizes the entire cervix and provides excellent diagnostic performance, even if only 2 excitation wavelengths are used (330 and 440 nm).[44] A pilot study reported that changes in MDC images were aligned with histologically defined CIN (cervical intraepithelial neoplasia).[62] A further study in 29 patients measured MDC specificity at 88% and sensitivity at 79% for differentiating cancerous lesions and HSILs from normal or LSILs.[46] Work to further optimize MDC performance is ongoing, and efforts to bring this cost-effective tool to developing countries have begun.[63,64]

The bedrock for properly evaluating device results is a meaningful gold-standard diagnosis. The research team established a robust qualitative histopathologic standard through careful assessment with a team of pathologists that evaluated thousands of cervical biopsy specimens.[65] A high level of agreement was achieved between evaluators, ensuring that reproducible comparisons could be made over the course of the study. Quantitative histopathologic measurements—nuclear morphology, chromatin texture, and DNA content—were also evaluated and will help to hone disease stratification.[30,66] The research team is proceeding to calculate means, medians, and ranges for feature data that have been collected from the thousands of consensus-read biopsies.

The research team has worked extensively to optimize quantitative histologic methodologies. A 3-tiered quality-assurance (QA) system for quantitative histology was developed based on data from a multicenter analysis of imaging results, with QA data derived from both short-term (daily) and long-term (semiannual) measurements.[67] Methodologic challenges—including variation in tissue section staining intensity, inter- and intraobserver variability of results, and the use of intermediate layer cells only—were also assessed in specimens collected from a large patient cohort (n = 1800).[66] The diagnostic performance of DNA ploidy mea-

surements of Feulgen-stained thin-preparation monolayer specimens were compared with conventional cytologic methods and the HC2 test.[68] Ploidy analysis was reported to have comparable sensitivity, specificity, and negative and positive predictive values compared with the other approaches, with this test having the added benefit of being semiautomated and requiring limited expertise with a quick turnaround for the results (within 2 days). To further decrease the turnaround time for such analyses, the research team has been working on a protocol using Azure A stain on cytologic specimens. This protocol may be completed in 2 hours, providing same-day results that would likely decrease the loss of patients to follow-up in developing and developed nations.[69]

OUTCOMES TO DATE

Intermediate Outcomes (Clinical Effectiveness)

Rigorous technology assessment requires well-designed trials. Over the course of this project, the research team has undertaken multiple pilot and Phase I/II studies. Through Phase II trials of quantitative cytology, the research team saw 1850 patients at 5 clinical sites, with this work confirming the feasibility of obtaining quick results by this technique.[68,70] Data from a Phase II trial in which >3500 cervical biopsies were evaluated using quantitative pathologic methodologies helped hone automated components of this technique and identify cell-level changes associated with different neoplastic stages.[66,67,71–73] The point probe for fluorescence and reflectance spectroscopy was also evaluated in a Phase II study on a similar number of tissue specimens—and these results were comparable to those obtained by other groups.[54,55,74,75] This investigation helped to identify the causes of measurement variability in the clinical setting (eg, differences in menopausal status), giving information essential for developing effective downstream normalization and analytical approaches. One pilot study based on the MDC reported that this device could be used effectively in a clinical setting and helped to establish operational parameters for larger studies that will follow (unpublished results [ClinicalTrials.gov identifier: NCT00513123]).

Upcoming trials for this project involve (1) evaluation of the previously mentioned technologies in expanded patient populations; (2) parallel analysis of these same technologies to determine whether combined application can improve diagnostic performance; and (3) evaluation of newer tools and device algorithms that have not yet been assessed in patient populations. The research team will evaluate the performance of the MDC in a clinical trial involving >600 patients. A primary goal of this work is the optimization of an algorithm for MDC data that can effectively delineate the presence/absence of disease compared to gold-standard histopathologic results. Another trial will evaluate 180 patients to determine whether combined application of the MDC and point probe devices leads to greater diagnostic accuracy.

Pilot trials are planned or have been initiated to assess the utility of other approaches for delineating cervical disease states. Based largely on feedback and ideas from Program Project members at the University of Ibadan in Nigeria, the research team has produced a device called the Diagnostic Imaging Aid (DIA). It is essentially a battery-powered, portable version of the MDC. This device will be assessed against standard approaches and other devices that the research team has developed during this project to determine whether it will have utility in developing countries (the DIA is less expensive to build, service, and use, and its portability ensures greater utility in resource-poor clinical settings). In vivo confocal microscopy, which has the capacity to produce real-time cell-level images of the cervical epithelium, will also be assessed in a pilot trial. Finally, a variety of contrast agents will be applied to the cervix to determine whether they can improve the detection of spectral changes associated with neoplastic processes.

Taken together, these clinical studies will provide a wealth of knowledge regarding the utility and practicality of a wide array of tools for detecting cervical disease, including which devices would be optimal for different clinical settings (eg, screening vs diagnostic populations, clinics in developed vs developing countries). With this work,

real-time diagnosis and treatment of cervical lesions are becoming viable. Furthermore, the improved specificities and sensitivities being obtained (eg, by improved instrumentation and diagnostic algorithms) are reducing the risk for undetected disease, limiting the possibility of overtreatment, and decreasing the clinical costs associated with managing this disease.

Patient and Provider Outcomes

The literature on technology assessment places a strong emphasis on evaluating the impact of new technologies on patient outcomes. These include assessments of physical, functional, or emotional well-being in patients after exposure to new technologies. Evaluating these patient outcomes during development phases can help to identify and fix problems before new technologies are disseminated. To ensure that analysis in this area is robust, patients must be of varied ages, drawn from ethnically diverse populations, and exhibit differences along additional socioeconomic measures (eg, marital status, education). Significantly, few studies have examined the impact of screening and diagnostic technologies on these outcomes.

The research team has attempted to integrate meaningful patient outcome evaluations into the clinical trials and pilot tests of the screening and diagnostic technologies. Based on patient feedback, the research team created and validated tools and approaches for measuring patient distress.[26,76–80] The findings suggest that patients who undergo screening and diagnosis perceive optical spectroscopy to be less painful than the Papanicolaou smear, colposcopy, and biopsy,[81] and that patients were less anxious during spectroscopy than during other tests.[77,82] When queried about their satisfaction after the examination, patients reported biopsy testing to be more frightening than spectroscopy. They also stated a preference for decreased lighting during spectroscopy. The only negative aspect of spectroscopy, as defined by the patients, was the extended amount of time needed to collect device measurements. Based on this feedback, study investigators involved in instrumentation have taken (and continue to take) steps to reduce the "time footprint" associated with spectroscopic measurements. Significantly, no long-term or short-term adverse events have been reported in these patients, suggesting the safety of study technologies.

The research team also assessed individual perceptions in addition to those associated with clinical screening.[83,84] For example, the research team queried patient knowledge regarding HPV and cervical dysplasia.[84] The limited knowledge evidenced by the results spurred the research team to produce new educational tools to better explain cervical cancer screening, diagnosis, and treatment. The research team also evaluated patient attitudes, behaviors, and barriers to participation in trials, screening, and treatments.[76,85,86] One finding from this work was that surveyed diagnostic and screening population patients rated test sensitivity as the most important test characteristic.[76] This same analysis suggested that some patients preferred not to receive same-day treatment following diagnosis, a finding that has prompted the group to develop tools that predict whether patients will want real-time disease management. Given its role in the adoption of new tools in clinical practice, knowledge dissemination was included as a direct goal in the Program Project design.[87,88] The group also conducted a study of health care providers' satisfaction with the device.[89] In this work, presented elsewhere in this issue, the primary obstacle to implementation in practice was the concern that a device capable of real-time diagnosis would lead to challenges regarding the duration and character of patient visits.

Societal Outcomes (and Economic Evaluation)

The research team measured the impact of the technology development on society in terms of health care costs, which can have significant effects in countries with limited resources, where the relative abundance of cervical cancer is directly related to the prohibitive expense of regular screening. The cost of diagnosing and evaluating atypias and LSILs in the United States is ~$6 billion per year.[90] With more effective screening and diagnostic tools, this substantial sum could be more effectively applied. For example, the billions

of dollars allocated to diagnose and evaluate cervical cancer could be used to reach underserved populations and fund better management of patients with disease that is more likely to progress.

To date, there have been few studies of the cost-effectiveness of emerging imaging technologies. This represents a lost opportunity, as economic considerations can have a positive impact on technology design. In the preliminary work for these optical spectroscopy devices, the research team found that only a minimal number of light wavelengths were biologically useful in the algorithm.[25,91] Identifying the ideal wavelengths of light allowed for the choice of a light source less expensive than a laser for a potential commercial device. (It also substantially reduced patients' exposure to UV light, although levels were already orders of magnitude below defined thresholds.) During this project, the research team devised an approach to estimate the diagnostic performance of Bayesian classifiers derived from optical spectra—and then used these results to evaluate the impact of variations in tissue type, sample size, patient population, and financial cost of the point probe device.[92] The net result of this work was a method for reducing experimental costs associated with the tools used for the diagnosis of cervical cancer that are based on optical spectra.

The group undertook several comparative analyses of different screening and diagnostic approaches. Specifically, they are evaluating the performance and cost-effectiveness of colposcopy, repeat Papanicolaou smear, endocervical curettage, point probe optical spectroscopy, MDC, combination point probe and MDC, an MDC algorithm, an MDC-point probe algorithm, a DIA device, VIA, and HPV testing.[55,74,75,90,93–100] These analyses are being undertaken in the United States and Canada, with similar studies planned for Nigeria. (Much of this work was mentioned earlier.)

This project has also provided new methodologies with applicability in other clinical trials. For example, the research team found that it was feasible to collect and analyze non–health care direct cost data (eg, costs associated with child/elder care or transportation/parking) and time cost data.[101] In this analysis, clinic type (community vs specialty hospital) and patient population (screening vs diagnostic) affected such costs. The findings also suggested that non-health care direct costs could be analyzed for a single large-scale trial, indicating that the approaches are widely applicable. The research team also developed an approach to quantitative pathology that uses measurements of 120 cell features on a section of tumor tissue to diagnose disease and guide patient-management decisions.[102] This approach makes use of a cumulative log–odds model score, followed by receiver operating characteristic (ROC) curve analysis, and is currently being evaluated in a larger patient cohort. In a third study, the research team evaluated previous cost–benefit ratio analyses for a variety of diseases, identifying ratio values associated with disease severity.[103] For example, directly life-threatening but curable clinical scenarios were found to have a cost–benefit ratio of <0.05. the approaches may have broad utility for guiding the selection of optimal test cutpoints on ROC curves during the development of diagnostic tests. Finally, the research team reviewed in detail the mathematical models being applied to the cervical cancer problem and discussed how they will affect research going forward.[100]

The natural history of cervical cancer was also evaluated during this project. The research team had previously evaluated the utility of intermediate markers as a means for guiding management of cervical lesions.[104] In this project, a meta-analysis was used for the determination of the risk for progression from HSIL to invasive disease and from LSIL to HSIL, and the probability of regression from HSIL to LSIL and from LSIL to normal.[105] This findings suggested that, although the risk for transition between cervical cancer stages may be small at half-year intervals, the cumulative risk of cervical cancer is significant. The research team also compared performance of the HC2 test and colposcopy versus the Papanicolaou smear in a large population, assessing the capacity of these approaches in the identification of disease in screening and diagnostic settings.[97,99] The group found that in women aged >30 years, the HC2 test was more effective for detecting disease in screening populations than the Papanicolaou smear.[97]

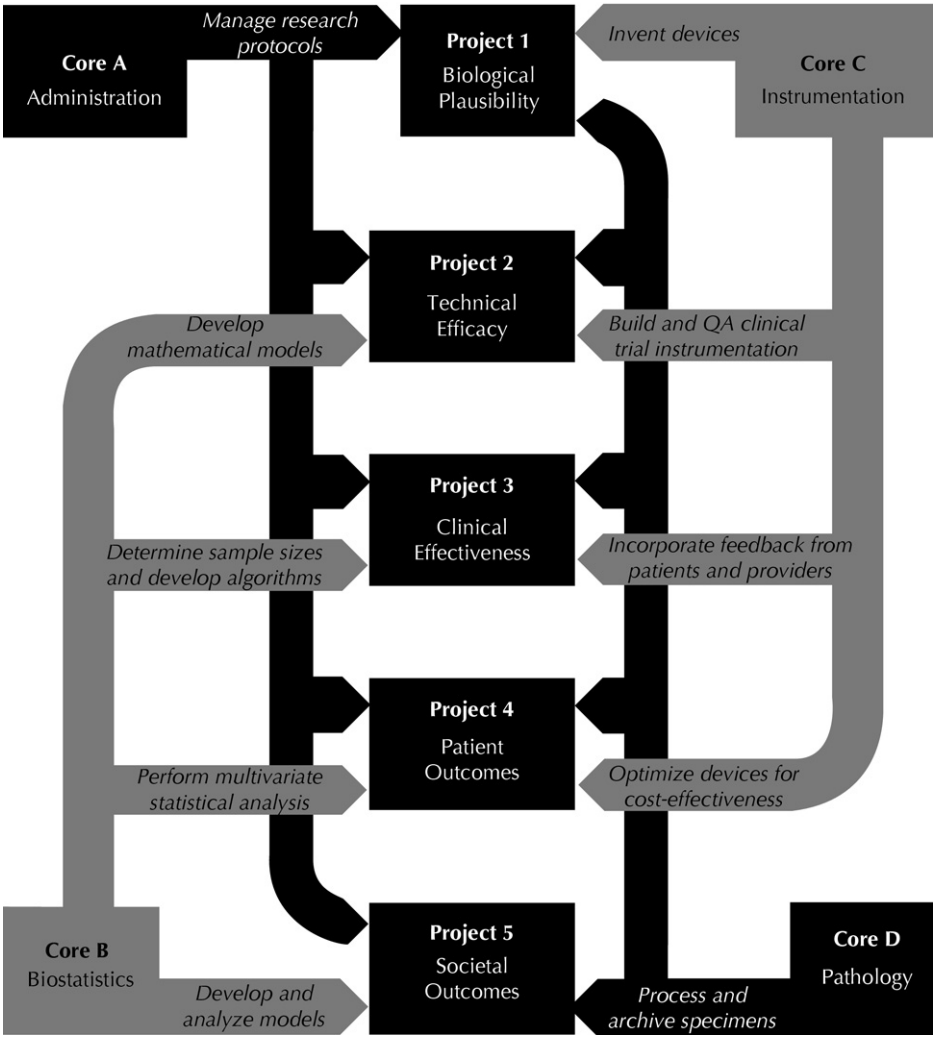

Figure 4. Relationship of project cores to the 5 subprojects of the wider program project endeavor. QA = quality assurance.

Colposcopy, on the other hand, was found to be more effective in diagnostic populations than in screening populations.[99]

Foundations (Project Cores)

The Program Project includes funding for resources and infrastructure that can support multiple research subprojects. Four "project cores" were defined for the work: an Administrative, Research Compliance, and Epidemiology Core; a Biostatistics and Data Management Core; an Instrumentation Core; and a Pathology and HPV Biomarkers Core. The many facets of technology development described earlier have been made possible by the existence of these effective support elements. The relationship between cores and subprojects is depicted in **Figure 4**.

Without the Administrative, Research Compliance, and Epidemiology Core, Nigeria could not have been secured as a major collaborator on this work, and patient diversity could not have been sufficiently tracked in clinical trials.[64,87,106–109] Novel disease-associated biomarkers could not have been derived, nor new insights into disease biology gleaned.[72,110–112] Weekly teleconference meetings by this group have helped to sustain productivity on this project, as have insights from the internal and external advisory boards associated with this work. The regular communication and interactions mandated by this project core have helped

to drive the successes detailed in this article by corralling individuals with disparate research interests and pointing them toward common goals.

The broad purview of the Biostatistics and Data Management Core has also enhanced this project over the past decade. Individuals associated with this core have done extensive work to address each of the technology development categories outlined earlier, including statistical analyses and algorithm development for several research components.* They have also worked closely with the other research cores associated with this project.† This group has also been responsible for the creation and proper maintenance of the secure patient database and the development of software and statistical approaches for analyzing this collected information.[102,122] Members of the Biostatistics and Data Management Core are currently developing new approaches to handle the multidimensional data sets and to facilitate the review and integrated analysis of spectral, quantitative cytologic, and quantitative pathologic data.

The Instrumentation Core has designed, built, calibrated, and maintained all of the devices used during the Program Project. Bioengineers at each project site have worked directly with health providers to track and improve device performance in real time. Dialogue with other project partners has helped to make tools more cost-effective, user-friendly, and patient-friendly. Specifically, the instrumentation core has built 3 point probe devices and developed and implemented quality assurance software for these tools[55,92]; developed 3 MDC devices and meaningful MDC QA metrics[63]; developed fiber optic probes for biomedical spectroscopic sensing[123,124]; and devised and executed trials to test the impact of intra- and interdevice variation on study measurements.[40,54–56,114,125]

Finally, inputs from the Pathology and HPV Biomarkers Core have been critical to the success of the different parts of this project. Having collected, processed, stained, and reviewed thousands of cervical biopsy slides, this group has provided the gold-standard, consensus-reviewed diagnoses that have been crucial to all components of this study.[65] All of the cytologic and histopathologic specimens needed for clinical and quantitative assessment have been collected and managed by this core. This core has also developed imaging systems for evaluating morphometric and architectural features on tissue cross-sections, with phenotypic scores calculated by this method correlating well with pathology classifications and HPV status.[48,66–68,70,73,120,121] Algorithms based on this approach have had particular utility in characterizing the underlying biology of cervical disease. Associations between HPV mRNA levels and specific dysplastic stages were also evaluated through the efforts of this core.[126,127]

CONCLUSIONS

This research initiative has spanned more than 10 years and 2 continents. It has resulted in nearly 200 publications, 2 dozen interinstitutional patents, and >50 graduate degrees and postdoctoral fellowships. More significantly, thousands of patients have been reviewed over its course, with hundreds of treatable cervical lesions identified in patient populations that may not have otherwise had access to effective screening. The imaging tools and approaches that the research team have developed for detecting cervical disease have steadily improved with respect to their accuracy, cost-effectiveness, and ease of use. Analyses that integrate the multiple levels of data the research team has generated are continuing to improve these parameters. The research team believes that the work will have a positive impact on the clinical and economic outcomes associated with cervical cancer, and that many of the organizational approaches and methodologies the research team has taken to this work could be used to augment other translational research projects, making collaborations more effective and sowing the seeds for greater innovation.

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CONFLICTS OF INTEREST

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Address correspondence to: Timon P.-H. Buys, PhD, BC Cancer Research Centre, 675 West 10th Avenue, Vancouver, BC, Canada V5Z 1L3. E-mail: tbuys@bccrc.ca