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TITLE: THE PAP SMEAR CHALLENGE: COMPARING CLINICAL PERFORMANCE OF A NOVEL "MOLECULAR PAP" BASED ON NEXT-GENERATION SEQUENCING TO TRADITIONAL CERVICAL CANCER SCREENING

PRINCIPAL INVESTIGATOR: COL JANE SHEN-GUNTHER

ORGANIZATION: BROOKE ARMY MEDICAL CENTER (BAMC)
Fort Sam Houston, TX 78234

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JANE SHEN-GUNTHER, MD, PHD, COL, MC, US ARMY

E-Mail: jane.shengunther.mil@mail.mil

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US ARMY BROOKE ARMY MEDICAL CENTER
BAMC)
ATTN: MCHE-CI
3698 CHAMBERS PASS
FORT SAM HOUSTON, TX 78234-6315

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14. ABSTRACT

Objective: The "Molecular Pap" is an integrated panel of biomarkers based on Human Papillomavirus (HPV) deep sequencing and quantitative DNA methylation of 3 human genes by pyrosequencing. To validate our biomarker panel and predictive model for Pap smear classification, we proposed to collect 700 Pap smear samples from each of 6 diagnostic categories for HPV genotyping and methylation quantification for direct comparison to traditional cervical cancer screening (cytology +/- cobas® HPV).

Methods: This prospective, cross-sectional study uses residual liquid-based cytology samples for HPV genotyping and epigenetic analysis by pyrosequencing. A total of 2,682 Pap samples have been collected to date and have undergone various stages of DNA extraction, HPV DNA amplification, Sanger and deep sequencing, and bioinformatics analysis.

Results: NA. Study is on-going.

Accomplishments (Year 1): Pap sample collection has reached 70% of target accrual and molecular analyses are in-progress. Four contracts for supplies, software, cloud-based bioinformatics and computing have been awarded. The development of a real-world, automated, data science pipeline for multiple types of genomics data has been initiated.

15. SUBJECT TERMS
Human Papillomavirus, HPV, HPV genotyping, Deep sequencing, DNA methylation, Pap smear, Pyrosequencing, Molecular diagnostics, Molecular biomarkers, Virome, NILM, ASCUS, LSIL, HSIL.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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1. **INTRODUCTION:** The "Molecular Pap" is an integrated panel of biomarkers based on Human Papillomavirus (HPV) deep sequencing and quantitative DNA methylation of 3 human genes by pyrosequencing. To validate our biomarker panel and predictive model for Pap smear classification, we proposed to collect 700 Pap smear samples from each of 6 diagnostic categories for HPV genotyping and methylation quantification for direct comparison to traditional cervical cancer screening (cytology +/- cobas® HPV). Concurrently, a bioinformatics pipeline for automated data collection, analysis, modeling and visualization via a cloud-based computing system will also be developed.
2. **KEYWORDS:** Human Papillomavirus, HPV, HPV genotyping, Deep sequencing, DNA methylation, Pap smear, Pyrosequencing, Molecular diagnostics, Molecular biomarkers, Virome, NILM, ASCUS, LSIL, HSIL.
3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

  Major goals of the project:

  a. CY17 Goals: Expand the applicability of the "Molecular Pap"
     i. Obtain IRB protocol approval.
     ii. Collect Pap smear samples from BAMC Cytopathology Laboratory.
     iii. Determine HPV genotype(s) by next-generation sequencing.
     iv. Quantify DNA methylation of 3 tumor suppressor genes.

  b. CY18-19 Goals: Compare clinical performance of the "Molecular Pap" to traditional Pap/HPV screening
     i. Use biomarkers & predictive modeling to classify Pap smears.
     ii. Compare "Molecular Pap" results to traditional Pap/HPV screening.
     iii. Implement bioinformatics pipeline via cloud computing.

- **What was accomplished under these goals?**

  Major activities accomplished:

  a. Research regulatory activities
     i. BAMC IRB protocol submitted: 07 MARCH 2017
     ii. BAMC IRB protocol approved: 09 JUNE 2017
     iii. ORP HRPO protocol submitted: 12 JUNE 2017
     iv. ORP HRPO protocol approved: 12 JULY 2017

b. Services contract activities
    i. Services contract for HPV next-gen sequencing (NGS) approved by MTF: 7 MAY 2017
        a) Services Contract awarded to **Lucigen Corporation** for HPV NGS (2,016 samples): 07 AUG 2017.
        b) POP: 15 AUG 17 TO 14 AUG 2018.

    ii. Laboratory Supplies Contract approved by MTF: 07 MAY 2017
        a) Laboratory Supplies Contract Awarded to **Government Scientific Source (GSS)**: 11 AUG 2017.
        b) POP: 21 AUG 2017 TO 20 AUG 2018.

    iii. Software Contract for NGS data analysis approved by MTF: 04 DEC 2017.
        a) Software contract awarded to **Qiagen Bioinformatics** for CLC Genomics Workbench & Microbial Genomics: 23 APR 2018
        b) POP: 25 APR 2018 TO 24 APR 2010.

    iv. Software & IT Services Non-disclosure Agreement (NDA) with Wolfram Solutions signed by Wolfram Research & USAMRMC Medical Tech Transfer: 17 NOV 2017.
        a) Software & IT Services contract to Wolfram Solutions for Cloud-based bioinformatics pipeline approved by MTF: 6 MAR 2018.
        b) Software & IT Services contract awarded to **Wolfram Solutions** for Cloud-based bioinformatics pipeline development and automation: 10 APR 2018.
        c) POP: 10 APR 2018 TO 9 APR 2019.

c. BAMC DCI Biomedical laboratory activities
    i. Pap smear samples collected to date (24 APR 18): N = **2,682**

    ii. Pap smear samples extracted of total DNA to date (24 APR 18): N = **2,136**

    iii. PCR for HPV DNA detection in genomic DNA derived from Pap samples to date (24 APR 18): N = **2,071**

    iv. Plates (96 samples/plate) sent for NGS to date (24 APR 18): N = **5** (successful results received)

    v. Plates (96 samples/plate) sent for Sanger sequencing to date (24 APR 18): N = **4**

vi. Optimizing pyrosequencing lab workflow on PyroMark Q48 instrument: on-going.

- **What opportunities for training and professional development has the project provided?**

Army active duty laboratory technicians (N= 3) and laboratory manager (N = 1) assigned to BAMC Dept. of Clinical Investigation have learned numerous molecular biology techniques, processes, instrumentation, and informatics as a results of this project. Furthermore, our two doctoral-level scientists (contract microbiologist & cancer biologist/experimental therapeutics) working on the current project have grown professionally by broadening their scope and knowledge in virology, oncology, genomics, deep sequencing, pyrosequencing, bioinformatics, and cloud-based computing.

- **How were the results disseminated to communities of interest?**

Nothing to Report

- **What do you plan to do during the next reporting period to accomplish the goals?**

a. Implement bioinformatics pipeline via cloud computing

i. Establish account with Amazon Web Services (AWS) GovCloud
ii. Establish bioinformatics workflow between AWS GovCloud S3 data storage and CLC Genomics platforms (CLC Genomics Cloud Engine, CLC Genomics Workbench (WB), and CLC Microbial Genomics plugin).
iii. Establish cloud-based data science workflow and analytics via Wolfram Solutions to include setup of real-world data science pipeline starting with curation of computable data, statistical analysis, predictive analytics, classification algorithms, and ending with results in written and graphical forms.

b. BAMC DCI Biomedical laboratory activities

i. Continue Pap smear sample collection to reach target accrual numbers.
ii. Continue HPV genotyping and pyrosequencing per protocol.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**

  Nothing to Report

- **What was the impact on other disciplines?**

  Nothing to Report

- **What was the impact on technology transfer?**

  USAMRMC Technology Transfer Office is currently working on licensing the *Molecular Pap*. Through their work, the *Molecular Pap* was selected as one of the 100+ cancer inventions to create high-impact startups through the Freedom from Cancer Startup Challenge in 2017-2018 (https://www.freedomfromcancerchallenge.org/). A team of scientists, entrepreneurs and business leaders from the University of Pennsylvania formed a team to pitch the *Molecular Pap* in this startup competition. The challenge is currently on-going. (See Appendix B for Press Release)

- **What was the impact on society beyond science and technology?**

  Nothing to Report

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**

  Nothing to Report

- **Actual or anticipated problems or delays and actions or plans to resolve them**

  Nothing to Report

- **Changes that had a significant impact on expenditures**

  Nothing to Report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

  None

- **Significant changes in use or care of human subjects**

  None

- **Significant changes in use or care of vertebrate animals.**

  NA

- **Significant changes in use of biohazards and/or select agents**

  NA

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**

  Nothing to Report

- **Journal publications.**

  Nothing to Report

- **Books or other non-periodical, one-time publications.**

  Nothing to Report

- **Other publications, conference papers, and presentations.**

  Nothing to Report

- **Website(s) or other Internet site(s)**

  Nothing to Report

- **Technologies or techniques**

  Nothing to Report

- **Inventions, patent applications, and/or licenses**

  The current project seeks to expand the applicability of the *Molecular Pap* to other cytological categories not previously tested. The molecular markers of the *Molecular Pap*, i.e. HPV genotype and *ADCY8, CDH8, and ZNF582* promoter methylation discovered in our previous project and the methods for Pap smear classification have been submitted to the World International Patent Organization (WIPO) for an international patent application (below). The application is currently in the second or "National" phase.

  Shen-Gunther J, inventor.
  United States Army, assignee.
  Title: Methods for molecularly characterizing cervical cell samples.
  United States Provisional Application 62/212,555. 2015 Sep 1.
  International Application Filing No. PCT/US2016/049426 2016 Aug 30.
  WIPO Pub. No. WO/2017/040491 Publication Date: 2017 Sep 03

- **Other Products**

  Biospecimen collections:

  - Genomic DNA derived from cervical cytology (N = 2,682)
  - HPV DNA of various genotypes derived from cervical cytology

  Research materials: unique PCR primers developed for pyrosequencing

  Bioinformatics: pipeline for HPV genotyping and metagenome analysis

  Data or databases:

  - HPV DNA sequences and genotyping data
  - Pyrosequencing data categorized by cervical pathology
  - HPV BLAST reference database created for CLC Genomics workbench

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Jane Shen-Gunther, MD, PhD, COL, MC, US ARMY
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-0876-9338
Nearest person month worked:	14
Contribution to Project:	Responsible for identifying patient records and samples applicable for study and de-identifying information; responsible for project oversight and coordination of all activities outlined in protocol and required IRB documentation. Conduct cellular and molecular testing and analysis of specimens (DNA extraction, PCR, next-generation sequencing (NGS), and pyrosequencing), data analysis and manuscript preparation. Coordinate development of bioinformatics pipeline with commercial entities and collaborators at UTSA and UTHSCSA.
Funding Support:	DoD US Army – Active Duty physician

Name:	Andrea Garcia, PhD
Project Role:	Sub-Investigator and Research Staff: Contract Biological Research Scientist with expertise in cancer/molecular biology, experimental therapeutics and bioinformatics
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	10
Contribution to Project:	Responsibilities: Cytology sample processing, cellular and molecular testing and analysis (DNA extraction, PCR, next-generation sequencing (NGS), and pyrosequencing), de-identified data analysis and manuscript preparation.
Funding Support:	Defense Health Program (DHP) Clinical Trial Infrastructure Support funds

Name:	Qingqing Xia, PhD
Project Role:	Sub-Investigator and Research Staff: Contract Biological Research Scientist with expertise in microbiology and molecular biology
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	7
Contribution to Project:	Responsibilities: Cytology sample processing, cellular and molecular testing and analysis (DNA extraction, PCR, next-generation sequencing (NGS), and pyrosequencing), de-identified data analysis and manuscript preparation.
Funding Support:	Defense Health Program (DHP) Clinical Trial Infrastructure Support funds

Name:	SPC Edetsira Kouame
Project Role:	Research Staff: Army Laboratory Technician
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	6
Contribution to Project:	Responsibilities: Assist with cytology sample processing, cellular and molecular testing and analysis (DNA extraction, PCR, next-generation sequencing (NGS), and pyrosequencing.
Funding Support:	DoD US Army – Active Duty Laboratory Technician

Name:	SPC Taylor Quickley
Project Role:	Research Staff: Army Laboratory Technician

Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	5
Contribution to Project:	Responsibilities: Assist with cytology sample processing, cellular and molecular testing and analysis (DNA extraction, PCR, next-generation sequencing (NGS), and pyrosequencing.
Funding Support:	DoD US Army – Active Duty Laboratory Technician

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  Nothing to Report

- **What other organizations were involved as partners?**

  Nothing to Report

  - **Organization Name**
  - **Location of Organization**
  - **Partner's contribution to the projec**t
  - **Financial support**
  - **In-kind support**
  - **Facilities**
  - **Collaboration**
  - **Personnel exchanges**
  - **Other**

8. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** NA
9. **APPENDICES:**
   - **Appendix A: QUAD CHART**
   - **Appendix B: TECHNOLOGY TRANSFER**

APPENDIX A. QUAD CHART

The Pap smear challenge: Comparing the clinical performance of a novel "Molecular Pap" based on next-generation sequencing to traditional cervical cancer screening.

PI: COL Jane Shen-Gunther **Org:** Brooke Army Medical Center Department of Clinical Investigation **Budget Amount:** $750,000

Study/Product Aim(s)

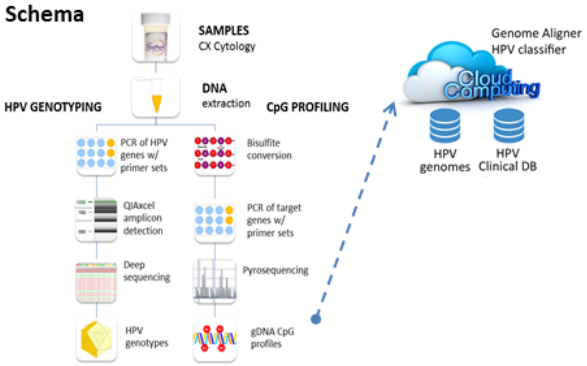
Aim 1: Expand the applicability of the "Molecular Pap" based on a panel of molecular biomarkers

Aim 2: Compare the clinical performance of the "Molecular Pap" to traditional Pap/HPV screening

Approach

The "Molecular Pap" is an integrated panel of biomarkers based on Human Papillomavirus (HPV) deep sequencing and quantitative DNA methylation of 3 human genes by pyrosequencing. To validate our biomarker panel and predictive model for Pap smear classification, we propose to collect 700 Pap smear samples from each of 6 diagnostic categories for HPV genotyping and methylation quantification for direct comparison to traditional cervical cancer screening (cytology +/- cobas® HPV). Concurrently, a bioinformatics pipeline for automated data collection, analysis, modeling and visualization via a cloud-based computing system will also be developed.

Schema



Timeline and Cost

Activities CY	16	17	18	19
Collect Pap smear samples for HPV next-gen sequencing & genotyping				
Quantify DNA methylation of 3 tumor suppressor genes.				
Compare "Molecular Pap" results to traditional Pap/HPV screening				
Develop & implement bioinformatics pipeline				
Estimated Budget ($K)	**$000**	**$250**	**$250**	**$250**

Updated: 7 September 2016

Goals/Milestones

CY16 – 17 Goals: Expand the applicability of the "Molecular Pap"

- Obtain IRB protocol approval
- Collect Pap smear samples from BAMC Cytopathology Laboratory.
- Determine HPV genotype(s) by next-generation sequencing.
- Quantify DNA methylation of 3 tumor suppressor genes.

CY18 Goals: Compare clinical performance of the "Molecular Pap" to traditional Pap/HPV screening

- Use biomarkers & predictive modeling to classify Pap smears
- Compare "Molecular Pap" results to traditional Pap/HPV screening.
- Implement bioinformatics pipeline via cloud computing.

Potential Challenges

- Bioinformatics pipeline development.

APPENDIX B. TECHNOLOGY TRANSFER

Wednesday, April 25, 2018 RSS

100+ Cancer Inventions Selected to Create High-Impact Startups through the Freedom from Cancer Startup Challenge

The Center for Advancing Innovation, the world's largest virtual startup challenge-based accelerator, announced today the 100+ breakthrough cancer inventions that will be commercialized through the Freedom from Cancer Startup Challenge (FCSC).

BETHESDA, MD (PRWEB) JULY 07, 2017

The Center for Advancing Innovation, the world's largest virtual startup challenge-based accelerator, announced today the 100+ breakthrough cancer inventions that will be commercialized through the Freedom from Cancer Startup Challenge (FCSC).

As CAI's largest challenge to date, the FCSC has the potential to create as many as 10,000 knowledge-based jobs and 100 oncology-focused startups. The Laura and John Arnold Foundation has committed $1.2 million in funding towards the FCSC. The challenge was also launched with support from MedImmune, the global biologics research and development arm of AstraZeneca.

The breakthrough inventions came from 55 institutions, including the National Institutes of Health (National Cancer Institute, National Heart, Lung, and Blood Institute, and National Institute of Biomedical Imaging and Bioengineering), the United States Army, and more than fifty American universities and hospitals. To identify these breakthrough inventions, CAI reviewed over 120,000 cancer-related patent applications, filed since 2005, and selected the top 1% from a total of 134 research institutions for deep dive due diligence. CAI then shortlisted the most promising 200 cancer-related inventions from a list of 400 technologies, made available for exclusive licensing by 63 research institutions that CAI engaged. From this short list, CAI and a 15-member FCSC invention selections committee cherry-picked 100+ commercially viable inventions to feature in the competition. Members of the selection committee included senior executives from MedImmune, Pfizer, and Novartis, as well as super angels, serial entrepreneurs, venture capitalists, and foundations.

Participating Technology Transfer Offices

> The intentional architecture and engineering of CAI's commercialization of cancer inventions in this challenge will serve to create a new paradigm to drive positive industry disruption, economic impact, and most importantly, social impact. - R. Truman

"CAI's track record in launching Life Sciences startups is outstanding and the FCSC initiative further establishes a new paradigm to kick start new cancer companies and is a force multiplier for the oncology ecosystem. CAI's systematic model establishes the trifecta necessary for high performing biotech companies - commercially viable inventions, rock star teams and funding - to get treatments to patients faster." - Jim Greenwood, BIO's President and CEO. Rosemarie Truman, Founder and CEO of CAI added, "The intentional architecture and engineering of CAI's commercialization of cancer inventions in this challenge will serve to create a new paradigm to drive positive industry disruption, economic impact, and most importantly, social impact."