AWARD NUMBER: W81XWH-18-1-0253

TITLE: A Rapid Blood Test to Differentiate Latent Tuberculosis from Active Disease

PRINCIPAL INVESTIGATOR: Antonino Catanzaro, MD

CONTRACTING ORGANIZATION: University of California, San Diego, La Jolla, CA

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Fort Detrick, Maryland 21702-5012

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, SAN DIEGO
OFFICE OF CONTRACT & GRANT ADMINISTRATION
9500 GILMAN DR, 0934
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of the study is to develop a blood-based TB test that meets or exceeds WHO Target Product Profiles for a rapid, biomarker-based, non-sputum triage test for detecting active TB disease. To accomplish this, activities in Year 1 included improvements to the 3-gene mRNA signature and analysis of these improvements; development of a 9-gene signature; prototype cartridge development; recruitment and blood collection in Moldova; and development of a secure data transmission system. In Year 2, Aim 2 recruitment and blood collection/processing was completed in Moldova, Cepheid worked to develop two "open" prototype cartridges – the Stanford 3-gene signature cartridge for non-stimulated blood, and a prototype antigen-stimulated cartridge. In Year 3, Cepheid completed biostatistics work necessary to lock the signatures and completed internal quality testing toward finalizing the prototype cartridges for field evaluation. The new field site in Pakistan was established and preparations to begin enrollment were completed. Prospective enrollment toward Aim 3 goals to evaluate the Cepheid cartridges will take place during the No-Cost Extension period (NCE).

15. SUBJECT TERMS
Tuberculosis, TB, mRNA signature, cartridge, triage test, blood test, finger stick, pre-clinical TB, active TB, latent TB, Moldova, WHO, TPP, biomarker-based

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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Unclassified	Unclassified	Unclassified	Unclassified	15	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION

The objective of this research is to develop a TB triage test which uses blood from a fingerstick that meets or exceeds WHO Targeted Product Profiles (TPP) for a rapid, biomarker-based, non-sputum triage test for detecting active TB. We plan to achieve this by developing an mRNA signature to discriminate patients with active TB from those with no TB, latent TB, or pre-clinical TB, validate this signature with prospectively collected blood from patients and contacts in the Republic of Moldova, transfer the signature into a Cepheid GeneXpert prototype cartridge, and then field test with a new cohort of prospectively enrolled patients suspected of having TB in Pakistan.

2. KEYWORDS

Tuberculosis, TB, mRNA signature, cartridge, triage test, blood test, finger stick, pre-clinical TB, active TB, latent TB, Moldova, WHO, TPP, biomarker-based.

3. ACCOMPLISHMENTS

What were the major goals of the project?

The major goal of the project is to develop a TB triage test using blood that meets or exceeds WHO Target Product Profiles for a rapid, biomarker-based, non-sputum triage test for detecting active TB disease (≥90% sensitivity when compared with the confirmatory test for active TB (both pulmonary and extrapulmonary) and ≥70% specificity against a microbiological reference standard. To accomplish this goal, our specific aims are 1) increase the power of our existing prototype mRNA signature to discriminate patients with active TB from those with no TB, latent TB or pre-clinical TB, 2) validate our improved TB signature using blood from 100 TB Index Cases and 450 household contacts in the Republic of Moldova and transfer the TB signature into the Cepheid GeneXpert prototype cartridge, and 3) field test the prototype cartridge with blood collected from 1,000 patients in Pakistan who are suspected of having TB.

The approved SOW also states site-specific tasks (for UCSD, Moldova, Cepheid, Stanford, the University of Arkansas, and Pakistan) to meet these project goals:

All Sites: Scientific collaboration, data analysis
Site 1 University of California, San Diego: obtain local IRB/IACUC Approval (UCSD Phase 1 IRB), and HRPO approval, IRB Phase 2
Site 2 Phthisiopneumology Institute and Public Association Society of Clinical Mycobacteriology, Chisinau, Republic of Moldova: obtain local IRB/IACUC Approval (UCSD Phase 1 IRB), and HRPO approval, IRB Phase 2, enroll patients, collect blood, testing prototype cartridge in Moldova
Site 3 Cepheid: Perform multiplex RT-PCR, run PAXgene & finger stick protocol, validate cartridge using blood, finalize prototype cartridge
Site 4 Stanford University: Discover active & latent TB signatures, validate active & latent TB signatures
Site 5 University of Arkansas: Discover active & latent TB signatures, validate active & latent TB signatures
Site 6 National TB Control Program of Pakistan: Enroll and test prototype cartridge in Pakistan

What was accomplished under these goals?

1) Major Activities: In Year 3 major activities of the project included additional participant enrollment at the Moldova field site to support cartridge development efforts at Cepheid, data cleaning and Aim

2 clinical database finalization at University of Arkansas, continued prototype cartridge development and optimization work at Cepheid, NanoString analysis on samples collected from Moldova to evaluate the analytical performance of the 9-gene and 3-gene signatures at Stanford, and critical start-up activities at the field site in Pakistan for the final field study. Each of these major activities is discussed in greater detail below.

Additional participant enrollment at the Moldova field site: During Year 3, two independent enrollment cohorts were recruited following completion of Aim 2 enrollment goals. The first cohort was completed in Q1 of Year 3 - we applied supplemental funds to enrich our clinical samples and help validate the down-selected signature to be used for Aim 3 field testing of the Cepheid stimulated prototype cartridge. We enrolled 55 microbiologically-confirmed, untreated TB positive patients and 128 contacts, approximately half of whom were uninfected and half of whom had LTBI by QuantiFERON reference testing. This number was sufficient to support a preliminary lock of the novel stimulated signature at Cepheid. These samples were shipped to Cepheid in early Q2. In Q3, an additional 25 active TB patients, 25 LTBI and 25 uninfected participants were also enrolled at the Moldova field site. The purpose of this final cohort was to de-risk the Aim 3 study in Pakistan by field testing and validating the specific sample stimulation peptides planned for use in the prototype cartridge based on antigen-stimulated blood. Additionally, this cohort included both QuantiFERON and LIOFeron LTBI testing in order to compare the performance of these assays. Clinical samples from this cohort were shipped to Cepheid in early Q4. Results of the analysis are pending.

Data cleaning and Aim 2 clinical database finalization at University of Arkansas: The data coordinating center worked to reconcile and clean clinical data collected from the Moldova field site. Efforts were increased as sample collection goals were met. A bi-weekly data reconciliation list was shared with the Moldova team to ensure missing forms and values were sent to the data center. Internal validation checks were also implemented to ensure high quality data within the final database, and the UCSD, University of Arkansas, and Cepheid teams met in Q3 for a final data-focused discussion to resolve outstanding data queries, and an updated dataset (version 6) was shared with Cepheid for analysis. Version 6 continues to be evaluated by the Cepheid team for final signature and cartridge development work.

Prototype cartridge development and optimization work at Cepheid: Cepheid's work in Year 3 was primarily focused on prototype cartridge development, optimization, and finalization for Aim 3 field testing. The project achieved a number of milestones during Year 3 toward these goals. First, samples collected in Moldova were collected using a GeneXpert-compatible lysis/stabilization buffer (PAXgene), which ensured that Cepheid had access to clinical samples for internal prototype cartridge verification before procuring the closed cartridges for field testing. Additionally, Cepheid implemented a 10-color format version of the prototype compatible with the GeneXpert instrument that launched commercially (CE-IVD) in 2020, instead of a marker configuration compatible with the legacy 6-color version of the current GeneXpert instrument install base, which gives Cepheid the option to include more markers with dedicated call-outs/Ct values. This increase chance of success for the two signatures (one for LTBI, one for active TB). Assay optimization work was conducted according to a simplified version of Cepheid's standard procedure used during Concept Phase and Technical Feasibility Phase with the goal of gaining time by focusing on the most critical aspects of product development. While the final goal is still to develop a single 10-color cartridge, Cepheid used two, 6-color prototype cartridges for antigen-stimulated blood for factorial experiments to further fine-tune the reagent compositions and optimize the GeneXpert program parameters for a robust assay with a shortened time-to-result. Later in Year 3, the two prototype cartridges were merged into one, containing Cepheid mRNA biomarkers, a reference gene and a Cepheid Sample Processing Control (SPC). The initial internal evaluation of this cartridge - Cepheid's first 10-color-, 2-signature-, 9-marker-, 2-control prototype cartridge for antigen-stimulated blood - was also carried out during

Project Year 3. The prototype cartridge classified the patients with nearly the same performance as singleplex qPCR using the 9 markers in the cartridge, for both the MTB Infected vs. Not Infected and for the ATB vs. LTBI signatures, respectively. Initial antigen stimulation protocol analysis results were in line with what has been seen earlier with the singleplex qPCR data set, with relevance for the Aim 3 field study protocol. Areas of improvement have been identified and will be implemented prior to production of the closed cartridges that will be sent for field testing in Pakistan in the NCE period.

NanoString analysis on collected samples to test the 9-gene and 3-gene signatures at Stanford: During Q2 of Year 3, a selection of PAXgene samples collected for the first *in vitro* performance testing of the novel 9-gene signature (developed analytically by Stanford) were shipped from Moldova to the US. The Stanford team coordinated the manufacturing and delivery of the novel 9-gene signature code sets to be analyzed by NanoString upon receipt of the extracted RNA samples. Preliminary theoretical analyses performed on data sources outside of the Moldova cohort showed that the 9-gene signature had the potential to meet the minimal WHO target product profile (TPP) of 90% sensitivity and 70% specificity for a TB triage test identifying patients with active TB. The 9-gene signature, developed specifically for this study to try and improve performance of the 3 gene signature, also showed a difference from the 3-gene signature in that 90% sensitivity was achieved earlier relative to specificity in this cohort: the 9-gene signature identifies contacts at risk of getting active TB up to 11 months before diagnosis. The 3-gene signature does this 6 months before diagnosis, allowing for earlier detection with the 9 gene signature, which could have substantial clinical impact. The Stanford study team then quantified the 9-gene mRNA signature in the 358 clinical samples from Moldova and presented analysis of the data to the UCSD group. This was the first prospective, *in-vitro* study of this novel signature developed for this study. In this cohort, at a minimum sensitivity of 90%, the specificity of the 9-gene signature was 67% while specificity for the 3-gene signature was 57%. These findings are major achievements toward study goals of increasing the power of our existing prototype mRNA signature to discriminate patients with active TB from those with no TB, and of meeting WHO TPP criteria for a rapid, biomarker-based, non-sputum triage test for detecting active TB disease. At the time of this report, final analyses are underway; results will be submitted for publication and shared with DoD officials when completed.

Start-up activities at the Aim 3 field site in Pakistan: In Year 3 we requested and received approval from DoD for a site change for the Aim 3 field study to the National TB Reference Laboratory (NTRL) in Islamabad, Pakistan. A budget and scope of work were also approved. Critical start-up activities that took place during Year 3 included equipment procurement and training for reference QuantiFERON testing, which is not included in routine testing at the NTRL. We also received approval from the local IRB for the Pakistan field site and from the UCSD IRB, and completed submission of these approvals for review by the DoD IRB; protocol development and training for field staff; study supply procurement; and case report form (CRF) development and training. In the process of implementing each of these important elements we have also gained a better understanding of the field conditions and have developed relationships with staff that will be working on the project in preparation for patient enrollment at this site.

2) Specific Objectives: The major activities in Year 3 supported the specific objectives outlined in the SOW for Year 3 including: discovery and augmentation of 3-gene TB signature, enrollment and blood collection in Moldova, prototype cartridge development, prototype cartridge component field testing, data analysis, and scientific collaboration.

3) Significant Results or Key Outcomes: A significant result of the Year 3 work was the development of a novel 9-gene signature and the first empiric testing and analyses finding its superiority in identification of active TB as compared to the previously established 3-gene signature. These findings will be critical to international goals of developing more accurate TB diagnostics to

help stop the spread of disease. Another key outcome of this project year was the final down-selection and locking of the antigen-stimulated signature for the Cepheid prototype latent TB cartridge (LTB cartridge) for field testing.

4) Other Achievements: Other major achievements were the final IRB approvals for the Pakistan field site and completion of preparation for enrollment of patients at the NTRL in Pakistan.

5) Stated Goals Not Met: The main goal in the SOW that was not met in Year 3 was prototype cartridge field testing. Due to the extraordinary obstacles and pressures resulting from the COVID-19 pandemic, we have been granted a 12-month no-cost extension to complete this work. This is discussed further in the plans for the next project year.

What opportunities for training and professional development has the project provided?
The project provided an opportunity for professional development and advancement of skills for laboratory technicians at the NTRL in Pakistan by providing QuantiFERON training via Qiagen. QuantiFERON testing is not included as part of routine testing at the NTRL, but will be included in this research. This training allows for technicians to conduct the required testing for the project, but also expands their technical skill set which should be a major benefit as Pakistan contemplates shifting to LTBI testing in the near future.

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?
During the next reporting period (Q1 of the 1-year NCE) we plan to initiate the Aim 3 field study at the NTRL in Pakistan. To accomplish this, UCSD will help to coordinate the shipment of the required GeneXpert research instruments, materials and reagents from Cepheid to Pakistan. UCSD will also conduct a final protocol training to include training on technical procedures for collection and processing of the Cepheid cartridges and collection and submission of study data. We also plan to submit a manuscript in collaboration with the Stanford study team with results of the 9-gene signature analysis.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
One of the most important potential impacts of our Year 3 work was in the area of predictive TB diagnosis. We completed the first empiric analysis of a novel 9-gene TB detection signature that indicates this signature performs better than the existing 3-gene signature and could potentially predict progression of TB from not infected or latent TB to active TB disease up to almost one year prior to the diagnosis. The 3-gene signature also has this feature, but has been shown to be predictive of disease only up to 6 months prior to active clinical disease. If these early results are verified and confirmed in large scale studies, it could lead to a powerful new clinical tool for diagnosing pre-clinical TB at a population level.

Additionally, preliminary data for the Cepheid antigen-stimulated cartridge in development indicates that this approach could potentially result in a novel product capable of detecting both LTBI and differentiating it from ATB, which is currently not possible with existing assays. We will evaluate this early data in a large clinical field study to determine if the early results can be reproduced. If confirmed, Cepheid could begin producing a novel product that has the potential to transform how LTBI and ATB are diagnosed at a global population scale.

What was the impact on other disciplines?
Nothing to Report

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
Nothing to Report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change
There were three main changes in approach for the study during Year 3. The primary change was the location of the field site for Aim 3 cartridge testing, from Moldova to Pakistan. This was approved in advance by DoD officials prior to implementation. The reason for this change was that an assessment of the impact of the COVID-19 pandemic in the region resulted in concerns about meeting critical field-testing sample size goals for Aim 3 in Moldova.

An additional change in approach during Year 3 was around the improvements to the 3-gene signature. We previously reported that the timeline of the project precluded production of a Cepheid cartridge that could be compatible with a 9-gene signature developed by Stanford. During Year 2, Stanford collaborators re-analyzed the data to see if any 4-gene signatures showed improvement compared to the current 3-gene signature as this would allow for optimal compatibility with the existing Cepheid 6-color cartridge. Ultimately, this analysis showed that none of the 4-gene signature combinations evaluated across 100 genes was significantly superior to the 3-gene signature. It was determined that the Cepheid cartridge would move forward with Cepheid markers/signatures using antigen-stimulated blood, while the 9-gene signature developed by Stanford would be evaluated and published to advance the science of TB diagnostics and inform future iterations of blood tests for TB.

Lastly, there has been a change in approach for the implementation of the LTB cartridge for field testing. A longer timeline than originally anticipated was given for finalizing and manufacturing the prototype LTB cartridge. This was identified as the main delay in beginning enrollment in Pakistan; therefore, UCSD and Cepheid agreed to an approach in which the enrollment team will collect blood for the LTB cartridge in antigen stimulation tubes, then freeze them on-site, and study staff will run them on-site once the prototype cartridges are delivered to Pakistan (estimated to occur during Q2). Meanwhile, enrollment will begin with the real-time testing of the existing 3-gene cartridge – enabling us to not only conduct testing of two different cartridges but also to compare performance of the two products. Once the prototype Ag stimulated cartridges are being delivered regularly to Pakistan, we will switch to running the stimulated and the 3 gene cartridges in real-time to also collect data on ease of use in real world conditions.

Actual or anticipated problems or delays and actions or plans to resolve them
At the time of this submission there are no existing problems or delays to report. The delays encountered have been addressed in the changes in approach discussed above. A potential issue that may arise is the need for additional funds. The approved NCE period is being carried out with the remaining funds from the initial project period. It is possible that this will not be adequate to support the full NCE to achieve project goals and we may encounter the need to request an additional supplement to complete the planned work as described in this report.

Changes that had a significant impact on expenditures
Slowdowns due to COVID-19 continued to delay expenditures related to both field and laboratory

research during this project year. The ongoing pandemic periodically restricted work at the UCSD and Cepheid laboratories, with work-from-home mandates and unpredictable case surges allowing us to continue project administration, but at times delaying progress on recruitment and laboratory-based research and testing. At the time of this report, with labs running at near-full capacity and COVID-19 case rates currently in decline, restrictions have eased and delays are less frequent.

Additionally, at the end of 2020 we initiated a request for a study enrollment site change from our Moldova lab to the National TB Reference Laboratory (NTRL) in Islamabad, Pakistan; this request was approved on 3/31/2021. While planning for the site change and awaiting agency approval, funds intended for the new site remained unexpended, causing a delay in the outgoing funding stream. Additionally, a longer timeline for finalizing and manufacturing the prototype LTB cartridge than originally anticipated further delayed start of enrollment in Pakistan. By the end of the reporting period these issues had been resolved, and enrollment in Pakistan was pending.

Finally, the financial management system change/replacement implemented at UCSD in July 2020, as described in our reports since that time, has continued to negatively affect the University's financial processing and reporting systems. Fortunately, over the last few months significant progress has been made, and reporting capabilities are steadily coming online. We anticipate continued improvements throughout the current project year.

Significant changes in use or care of human subjects
Nothing to report.

Significant changes in use or care of vertebrate animals
Nothing to report.

Significant changes in use of biohazards and/or select agents
Nothing to report.

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
Under site PI, Dr. Purvesh Khatri, the Stanford team has filed a patent application for the 9-gene signature. Approval is pending at the time of this report.

Other Products
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Antonino Catanzaro, MD
Project Role:	Project PI
Nearest person month worked:	2
Contribution to Project:	No change

Name:	Timothy Rodwell
Project Role:	Co-Investigator, UCSD
Nearest person month worked:	1
Contribution to Project:	No change

Name:	Peter Chiles
Project Role:	Laboratory Manager, UCSD
Nearest person month worked:	4
Contribution to Project:	No change

Name:	Laura Myhovich
Project Role:	Project Coordinator, UCSD
Nearest person month worked:	5
Contribution to Project:	No change

Name:	Jennie Hermansson
Project Role:	Research Scientist, Cepheid
Nearest person month worked:	11
Contribution to Project:	Data analysis and marker down-selection; Prototype Xpert Latent TB assay development

Name:	Antonio Ascue Avalos
Project Role:	Research Scientist, Cepheid
Nearest person month worked:	11
Contribution to Project:	Data analysis and marker down-selection; Prototype Xpert Latent TB assay development

Name:	Sarah Tidström
Project Role:	Senior Scientist, Cepheid
eRA Commons ID:	n/a
Nearest person month worked:	11
Contribution to Project:	Data analysis and marker down-selection; Prototype Xpert Latent TB assay development

Name:	Jonathan Siegrist
Project Role:	VP Innovation, Cepheid
Nearest person month worked:	1 (funded by Cepheid)

Contribution to Project: No change
Name: Ellen Wallace
Project Role: Assay Development Lead, Cepheid
Nearest person month worked: 6 (funded by Cepheid)
Contribution to Project: Innovation Bio R&D

Name: Purvesh Khatri
Project Role: Stanford Site PI
Nearest person month worked: 2
Contribution to Project: No change

Name: Michele Donato
Project Role: Postdoc, Stanford
Nearest person month worked: 1
Contribution to Project: No change

Name: Alex Skrenchuk
Project Role: Systems Administrator, Stanford
eRA Commons ID: n/a
Nearest person month worked: 1
Contribution to Project: No change

Name: Donald Catanzaro
Project Role: University of Arkansas Site PI
Nearest person month worked: 4
Contribution to Project: No change

Name: Maryam Kheirandish Borujeni
Project Role: Graduate Student, University of Arkansas
Nearest person month worked: 3
Contribution to Project: No change

Name: Valeriu Crudu
Project Role: Moldova Site PI
Nearest person month worked: 1
Contribution to Project: No change

Name: Elena Tudor
Project Role: Clinical Coordinator, Moldova
Nearest person month worked: 2
Contribution to Project: No change

Name: Mariana Macari
Project Role: Administrator
Nearest person month worked: 1
Contribution to Project: Finance management

Name: Alexandru Codreanu
Project Role: Laboratory Assistant, Moldova
Nearest person month worked: 3
Contribution to Project: No change

Name: Nelly Ciobanu
Project Role: Laboratory Coordinator, Moldova
Nearest person month worked: 4
Contribution to Project: No change

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

There are no categorical changes (e.g. from Pending to Current, or Current to Completed) to report since the last provision of Other Support information (October 2020). One addition to Other Support for UCSD Co-Investigator Dr. Rodwell is provided below (omitted in error from previous report)

RODWELL, TIMOTHY (UCSD Co-Investigator)

R01AI131939 Metcalfe (PI) 01/19/2018 - 12/31/2022 5% effort
NIH/NIAID (UCSD sub only)
Novel next-generation sequencing assay for monitoring multidrug resistant tuberculosis treatment in the setting of HIV infection
This project proposes to test the hypothesis that Quantitative low-level Mtb heteroresistance willcorrelate with intensive pharmacokinetic measurements and that inc orporation of SMOR-defined fluoroquinolones and aminoglycoside-resistant subpopulations with conventional predictors will predict subsequent phenotypic resistance earlier than LPA-defined resistant subpopulations.
Role: Sub-award site PI

What other organizations were involved as partners?

Organization Name:	**University of Arkansas**
Location of Organization:	Fayetteville, Arkansas
Partner's contribution to project:	a) Facilities (PI office space, data core facilities) b) Collaboration
Organization Name:	**Stanford University**
Location of Organization:	Stanford, California
Partner's contribution to project:	a) Facilities (PI office space, computational biology and translational medicine research laboratory space) b) Collaboration
Organization Name:	**Cepheid**
Location of Organization:	Solna, Sweden
Partner's contribution to project:	a) In-kind support (PI salary paid by Cepheid) b) Facilities (PI office space, R&D and manufacturing facilities) c) Collaboration
Organization Name:	**Institute of Phthisiopneumology**
Location of Organization:	Chisinau, Moldova
Partner's contribution to project:	a)Facilities (PI office space, Microbiology & Morphology laboratory) b) Collaboration
Organization Name:	**Public Association Society of Clinical Mycobacteriology from Republic of Moldova**
Location of Organization:	Chisinau, Moldova
Partner's contribution to project:	a) Collaboration

Organization Name:	**National TB Control Program**
Location of Organization:	Islamabad, Pakistan
Partner's contribution to project:	a) Facilities (PI office space, National TB Reference Laboratory [NTRL]) b) Collaboration

8. SPECIAL REPORTING REQUIREMENTS

Award Chart (Page 11) and Quad Chart (Page 12*)*

PR171076: A Rapid Blood Test to Differentiate Latent Tuberculosis from Active Disease

PI: Antonino Catanzaro; University of California, San Diego; California **Budget:** $3,570,150

Topic Area: PRMRP-TTDA **Mechanism:** W81XWH-17-PRMRP-TTDA

Research Area(s): Tuberculosis **Award Status:** September 30, 2018 – September 29, 2022

Study Goals:

The major goal of the project is to develop a TB triage test using blood that meets or exceeds WHO Target Product Profiles for a rapid, biomarker-based, non-sputum triage test for detecting active TB disease (≥90% sensitivity when compared with the confirmatory test for active TB (both pulmonary and extrapulmonary) and ≥70% specificity against a microbiological reference standard.

Specific Aims:

1) Use bioinformatics on our database of RNA expression to select genes which increase the robustness and performance of our mRNA signature to discriminate active TB, pre-clinical TB, and healthy, uninfected individuals
2) Validate our TB signature using blood from TB index cases & their contacts in the Republic of Moldova; transfer the TB signature into the Cepheid GeneXpert prototype cartridge
3) Field test the prototype cartridge in Pakistan with a new cohort of prospectively enrolled patients suspected to have TB

Key Accomplishments and Outcomes:

Publications: Hayley Warsinske, Rohit Vashisht, Purvesh Khatri. Host-response-based gene signatures for tuberculosis diagnosis: a systematic comparison of 15 signatures. PLoS Medicine 2019, 16(4):e1002786.

Patents: none to date (pending)

Funding Obtained: $3,570,150

A Rapid Blood Test to Differentiate Latent Tuberculosis from Active Disease

PR171076

W81XWH1810253

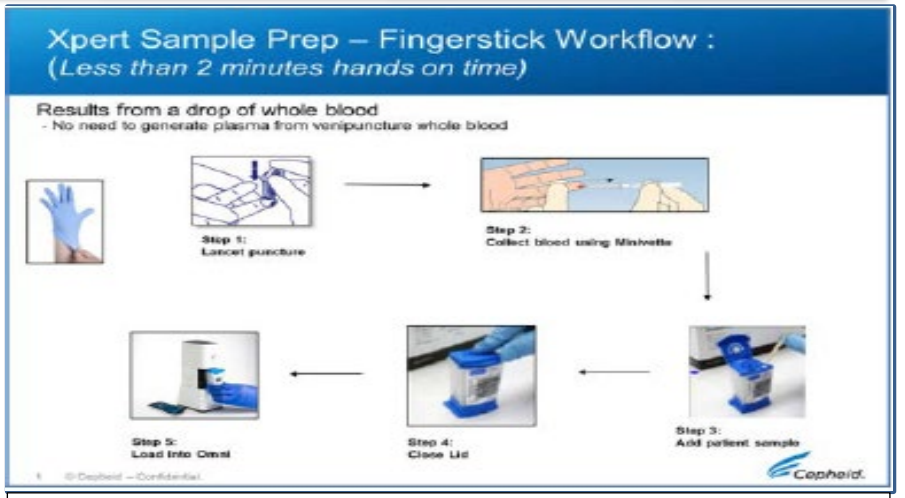
PI: Antonino Catanzaro, MD **Org:** The Regents of the University of California, San Diego **Award Amount:** $3,570,150

Study/Product Aim(s)

1) Use bioinformatics on our database of RNA expression to select genes which increase the robustness and performance of our 3-gene signature to discriminate active TB, pre-clinical TB, and healthy, uninfected individuals
2) Validate our TB signature using blood from TB index cases & their contacts in the Republic of Moldova; transfer the TB signature into the Cepheid GeneXpert prototype cartridge
3) Field test the prototype cartridge using blood collected in Pakistan from 1,000 individuals

Approach

For Aim 1, we will apply our computational framework for integrated multi-cohort analysis of gene expression data to pre-collected datasets (which include profiled patients with latent Mtb infection, along with healthy controls, and patients with active TB or other diseases), utilizing the WHO TB Diagnostics Development framework for test development. In Aims 2 & 3, we will recruit TB Index Cases from our clinical study site. Nurses will conduct epidemiological contact investigations to identify transmissions of TB to a close contact. Bloods will be collected and tested, first for improvement of the prototype (Aim 2), then for cartridge validation (Aim 3).



Accomplishments: Completion of enrollment in Moldova, down-selection & signature lock for Cepheid prototype cartridge; *in-vitro* performance testing of the novel 9-gene signature developed by Stanford; site change and start-up activities for new field site in Pakistan.

Activities CY	18	19	20	21	22
1. Discovery & Augmentation of 3-gene TB signature					
2. Validation					
3. Enrollment & blood collection in Moldova					
4. Prototype cartridge development					
5. Field Trial in Pakistan					
Estimated Budget ($K)	**56,410**	**376,361**	**1,258,096**	**1,217,884**	**661,399**

Updated: 11/01/2021

Goals/Milestones

CY18 Goal – Project Initiation & Study Partner Engagement

☑ Scientific collaboration

CY19 Goals

Discovery/Augmentation/Validation of 3-gene TB signature

☑ Discovery & Validation of Active & Latent TB Scores

Enrollment & Blood Collection in Moldova

☑ Obtain local IRB/IUCAC approval (IRB Phase 1) & HRPO approval

☑ Enroll patients, collect blood

CY20 Goal – Prototype Cartridge Development

☑ Perform RT-PCR; Run PAXgene & finger-stick protocol

☑ Validate cartridge

CY21-22 Goal – Field Trial in Pakistan

☐ Finalize prototype cartridge; field test at clinical site

Comments/Challenges/Issues/Concerns: Subaward liens as of 9/29/21 = $500,094 not included in Actual Expenditure amount below

Budget Expenditure to Date

Projected Expenditure: $3,570,150

Actual Expenditure: $2,688,284