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 92093-0934

REPORT DATE: October 2020

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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A Rapid Blood Test to Differentiate Latent Tuberculosis from Active Disease

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W81XWH-18-1-0253

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6. AUTHOR(S)
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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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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
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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 -- and Stanford began discovery analysis toward a sub-9-gene signature using a machine learning framework. In Year 3, Cepheid will further develop the"open" prototype cartridges, complete biostatistics work necessary to lock the signatures, and assess and validate their performance; the result will be a final "closed" prototype cartridge which will be deployed in the field.

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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1. INTRODUCTION

The objective of this research is to develop a TB triage test which uses blood from a finger-stick 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 the Republic of Moldova.

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 the Republic of Moldova who are suspected of having TB.

The approved SOW also states site-specific tasks (for UCSD, Moldova, Cepheid, Stanford, and the University of Arkansas) 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

What was accomplished under these goals?

1) **Major activities:** In Year 2 the major activities included completion of participant enrollment and blood collection/processing in Moldova for down-selecting final antigen-stimulated host biosignatures at Cepheid and validating prototype latent TB cartridges, as well as discovery and validation work for a sub-9-gene signature at Stanford, and broad scientific collaborations. Despite major obstacles encountered as result of the COVID-19 pandemic and global health emergency, we were able to continue many study activities. Due to obstacles encountered related to the pandemic, in Quarter 4 we applied for and received a budget supplement to help make up for lost time and to add an additional supplemental sample collection in Moldova. The supplement was granted and allowed for, among other things, re-starting recruitment in Moldova to collect specific cohorts to validate the down-selected signature for Aim 3 field testing of the Cepheid prototype cartridge.

   Participant enrollment and blood collection/processing: During Year 2 of the project, enrollment and collection of samples from TB index cases and their close contacts was completed according to Aim 2 enrollment targets (113 index cases and 454 close contacts). In this project year, follow-up visits continued as much as safely possible during the pandemic. The laboratory in Moldova continued to run QFT-plus in real-time and results were shared with the data center at University of Arkansas. With the awarded supplement, Moldova was able to re-initiate enrollment to meet the goals of recruiting an additional 25 not-infected contacts, 25 with latent TB, and 25 index cases. Cepheid's analyses indicated that time on treatment was a significant factor in the prototype cartridge validation; therefore, the index case enrollment was targeted such that only patients with <5 days of treatment (or ideally untreated) were enrolled. This work will continue into early Year 3 but as of this report it is progressing steadily, and we anticipate no problems with the Moldova team completing this.

   Discovery work for a sub-9-gene signature: The Stanford laboratory has been severely limited during the pandemic as the University imposed restrictions on research not directly related to COVID-19; however, the PI Dr. Khatri and his team, were able to continue *in silico* work. As reported in Year 1, Stanford completed *in silico* work to develop a more complex mRNA signature of 9 genes. This 9-gene signature met and exceeded minimum WHO TPP criteria as well as optimum criteria (95% sensitivity, 80% specificity) for distinguishing patients with active TB disease from healthy controls (observed performance 95% sensitivity, 83% specificity). Despite this, Cepheid determined that a 9-gene cartridge was unrealistic in the study budget and timeframe. Therefore, in Year 2 the Stanford team began discovery analysis toward a sub-9-gene signature using a machine learning framework. In this process, the goal of a high sensitivity/specificity 4-gene signature was established. After performance analysis of approximately 100 gene combinations *in silico*, none of these 4-gene signatures indicated improved performance over the 9-gene or 3-gene signatures for discrimination of active TB, latent, pre-clinical TB, and healthy uninfected individuals. NanoString primers for the 9-gene RNA code set were designed and

manufactured during Year 2. The research team decided to move forward with testing the Moldova samples with this code set to conduct *in vitro* testing of the 9-gene signature and subsets, including the 3-gene signature and multiple 4-gene candidates. Because the Stanford laboratory was unable to perform RNA extraction from the PAXgene tubes collected for the study due to COVID-19 restrictions, UCSD began plans to find an alternate lab to extract the RNA and send to NanoString. Arrangements for samples to be shipped from Moldova to the US were initiated in late Year 2.

Progress toward prototype cartridge development: During Year 2, Cepheid performed qPCR analysis on QFT lysates and PAXgene samples received from Moldova for the antigen-stimulated signature validation. In July 2020 at the quarterly All-Hands webinar, Cepheid presented the analyses of the main 15 candidate markers and signatures for antigen-stimulated blood. As clinical data became available from Moldova, Cepheid was provided with data to support signature lock-down. Cepheid worked during Year 2 to develop two open prototype cartridges – one that is antigen-stimulated, and one with the 3-gene host response signature. These two prototype cartridges are "open" such that they can be run with liquid PCR reagents ("wet" master mix format), as well as with lyophilized reagent beads (more similar to a final Cepheid product). The eight markers in the two cartridges are a promising subset of a 16-gene candidate set.

Scientific collaboration: In Year 2, the quarterly All-Hands calls continued with each collaborator joining and giving an update on progress, barriers, and plans for the following quarter. During Year 2 the All-Hands calls stimulated the need for further discussions, and ad hoc calls were scheduled to give opportunity for additional scientific collaboration. Additionally, the weekly webinars with PI Dr. Valeriu Crudu and the project staff in Moldova continued throughout Year 2. These calls were instrumental in monitoring study progress, data quality assurance, and laboratory processing, particularly in quarters 3 and 4 when the COVID-19 pandemic severely impacted participant-related study activities.

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

3) **Significant Results or Key Outcomes:** Year 2 was impacted directly and significantly by the COVID-19 global health emergency. Despite this, the project maintained forward progress toward stated goals, but did not have any significant results or key outcomes to for Year 2 additional to what is reported in section 1 above.

4) **Other Achievements:** There were additional achievements during this project year, the first being implementation of a centralized data sharing pipeline with the establishment of the UCSD OneDrive. This resource has improved integration of the laboratory results from Cepheid and the clinical data collected from Moldova by the University of Arkansas data core. This will lead to better communication and collaboration with study data. There were also notable advances in Year 2 in the conceptual understanding of the nuances of the spectrum of TB disease (discussed further in section 4 *Impact*). Additional achievements

were completion of proposed study enrollment, and regular monthly delivery of samples from Moldova to Cepheid (until the COVID-19 pandemic prevented continuation of this schedule).

5) **Stated Goals Not Met:** As of the end of Year 2, the goals stated in the SOW that were not met were to validate our 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 prototype cartridge. This work is actively underway; the samples have been sent to Cepheid and processed, and with the achievement of the centralized data sharing pipeline, we will have a chance to analyze the data to achieve this goal in the upcoming quarter.

What opportunities for training and professional development has the project provided?
During Year 2, an opportunity for professional development was provided by the project to research staff in Moldova. A registration link was circulated for a webinar via Rutgers Global Tuberculosis Institute (GTBI). The webinar, which took place on August 25, 2020, was a TB Nurse Case Conference entitled “Battle of the (Respiratory) Stars: TB vs. COVID-19”, provided jointly by the Tuberculosis Centers of Excellence for Training, Education, and Medical Consultation and the National Tuberculosis Controllers Association. This webinar applied directly to the ongoing impact of concurrent TB and COVID-19 outbreaks in Moldova and had bearing on both general professional development and to the project, as staff worked to balance this shifting public health environment.

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 (Year 3), the following activities are planned at each site:

UCSD/Moldova/University of Arkansas: UCSD, in collaboration with Moldova and the University of Arkansas plans to complete the collection of the additional 75 participant samples in Moldova needed to validate the prototype cartridges (25 active TB with <5 days on treatment, 25 not infected contacts, and 25 latent TB contacts). We will send these samples and associated clinical data to Cepheid early in Year 3. Additionally, we will design the Aim 3 field trial protocol; secure UCSD, University of Arkansas, field site, and DoD IRB approvals; and initiate the field trial to test the cartridge.

Stanford: In Year 2, Stanford succeeded in developing an improved signature with the 9-gene signature (sensitivity and specificity reported in section 3 *What was accomplished under these goals?*). Because Cepheid determined they were unable to develop the cartridge for the 9-gene signature within the study budget and timeframe, they moved forward with the antigen-stimulated approach. Meanwhile, in Year 3, the Stanford team will pursue *in vitro* validation of the 9-gene signature by analyzing RNA extracted from PAXgene tubes collected in Moldova with the NanoString 9-gene code set, analyze results for the 9-gene signature, and report the findings to the study team. These results will be compared to the existing 3-gene signature to determine if the larger signature yields better performance. If warranted, further analyses can

be completed on smaller gene sets within the 9-gene signature to explore the potential of 4-gene signatures to add performance value over the existing 3-gene signature.

Additionally in Year 3, Stanford will perform quality assurance testing for the 3-gene signature in the Cepheid cartridge using extracted RNA from PAXgene tubes collected from patients enrolled in the Aim 3 field trial.

Cepheid: During Year 2, Stanford presented this study with analysis from other studies that suggest that the 3-gene signature performs even better than initially thought. Therefore, Cepheid plans to move forward in Year 3 with two cartridges: the Stanford 3-gene signature cartridge for non-stimulated blood, and with the prototype antigen-stimulated cartridge. Cepheid will then complete biostatistics work necessary to lock the signatures, assessing performance of the prototype cartridge in relation to existing TB negative and latent TB assays and to applicable TB guidelines including the WHO 2020 "WHO consolidated guidelines on tuberculosis: tuberculosis preventive treatment". Cepheid will also validate the two "open" prototype cartridges using Close Contact follow-up samples previously collected and the additional set of 75 participant samples. The GeneXpert cartridge development work to be done during Year 3 will include a *single prototype GeneXpert cartridge assay strategy*; i. e. a 10-color format compatible with the GeneXpert instrument that launched commercially (CE-IVD) in 2020 (preferred option), or compatible with the 6-color version of the current GeneXpert instrument installed base (back-up option). This assay will be a highly-complex, multi-marker, 2-signature GeneXpert cartridge, in addition to being Cepheid's first antigen-stimulation assay. This will result in a final "closed" prototype cartridge for field deployment. All assay development work will be subject to Cepheid's internal verification studies to ensure high quality for the field trial.

Toward Aim 3 goals, Cepheid will support UCSD in preparations for the field study and collaborate on the patient cohort suitable for the assay under evaluation; manufacture up to 1,000 prototype cartridges to be field tested; deliver the assays to the clinical site; and provide necessary training and support to study staff.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
The principle discipline we had impact on in Year 2 was TB diagnostics science. As this project has progressed, so too has our conceptual understanding of the nuances of the spectrum of TB disease. Legacy TB diagnostics were primarily concerned with the detection of patients that did or did not "have" clinical TB disease. As we have embraced more sophisticated modalities for TB diagnosis – such as the host biomarkers used in this study – we have had to adopt a more nuanced understanding of the clinical stages of TB infection and disease. The product we plan to develop and test in this study is an IVD that can distinguish active TB from latent TB and no TB infection or disease. This required us to also develop more sophisticated means of categorizing TB patients and has resulted in more complex TB patient sample collection and data processing workflows. While this has introduced obstacles, it has also given us an opportunity to improve our means of defining reference lab methods and results.

What was the impact on other disciplines?
We feel that the methods we have adopted for reference sample categorization, to help us properly understand the diversity of host biomarker signatures we are observing, while specific to TB in this study, could also have an impact on other diseases with similarly complex, multi-stage natural histories.

What was the impact on technology transfer?
Our methods for clinically categorizing patients acknowledge and incorporate the disease complexities of TB instead of ignoring them. This has allowed Cepheid to understand the complexity and diversity of host biomarker responses they are observing and will support the eventual down-selection of signatures to the lowest optimal number, which will have positive downstream impacts on product design, cost and implementation.

What was the impact on society beyond science and technology?
Nothing to report in Y2

5. CHANGES/PROBLEMS

Changes in approach and reasons for change
In the Year 1 annual report, we reported that we were exploring the possibility of a sub-9-gene signature, as the time remaining in this project precludes production of a cartridge that can evaluate a 9-genes. In Quarter 1 of Year 2, we updated that the Stanford collaborators would re-analyze the data and provide Cepheid with up to 25 different 4-gene signature options across 100 genes which will be analyzed for iterative improvement compared to the current 3-gene signature as well as optimal compatibility with the existing Cepheid 6-color cartridge. This work was ongoing throughout Year 2. Though not a change to the overall approach, we report it here as an update on the status of these plans stated in the Year 1 annual report. Cepheid continues to work on the antigen-stimulated prototype cartridge as previously introduced.

An additional change to report is that this project was granted supplemental funding to complete the signature-lock-down work and get the project back on the planned trajectory following COVID-19-related delays.

Actual or anticipated problems or delays and actions or plans to resolve them
The problems encountered during Year 2 were related primarily to slowdowns caused by the COVID-19 pandemic and local restrictions at each of the collaborating sites. There were additional burdens on PIs of all collaborating sites, work reductions due to laboratory capacity, and reduced patient interactions.

During Year 2 there were also ongoing delays in providing clinical data associated with samples to partners at Cepheid, both unrelated to COVID-19, and then related to reduced work capacity in Moldova due to the pandemic. Although significant progress has been made, this is still included as a delay as it is not yet fully resolved. The data team at University of Arkansas has committed to sharing a dataset in the first month of Year 3, and follow-up calls to discuss any questions about the data will be scheduled immediately after. Because of this, we

anticipate delays in locking the signatures at Cepheid, which will subsequently delay production of the prototype cartridges and initiation of a field trial. The plans to resolve this are to utilize the supplemental funding to engage additional staff and ramp up activities to meet targeted timelines. Additionally, a data sharing plan has been created and agreed to by all parties between UCSD, Cepheid, and University of Arkansas.

Changes that had a significant impact on expenditures
Slowdowns due to COVID-19 delayed expenditures related to both field and laboratory research during the final 7 months of this project year. The pandemic placed severe restrictions on work at the UCSD and Cepheid laboratories, with work-from-home mandates allowing us to continue project administration, but delaying progress on laboratory-based research and testing. With patient recruitment temporarily shut down in Moldova, expenditures related to patient recruitment, sample processing and testing at that field site were also delayed. Recognizing that our timeline for Years 2 and 3 deliverables could be negatively impacted due to the effects of the shutdown, we submitted a request in July 2020 for supplemental funding, which this agency generously granted. With this additional funding, recruitment once again underway, and the gradual re-opening of our labs and research spaces, we are again making good progress toward our timeline and goals.

Additionally, in July 2020, the University of California San Diego replaced its legacy financial management system with Oracle Enterprise Resource Planning (ERP) Cloud. The switchover has resulted in delays in financial updates and reporting, the processing of outgoing sub-award modifications and payments, and real-time adjustments/updates to charges for UCSD personnel/effort. Though the new system is “live”, we are still in a transitional phase in which financial reporting and processing continue to be delayed. We anticipate measurable improvements to these systems by early 2021.

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; 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
Nothing to 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:	Malin Nygren
Project Role:	Cepheid Site PI
Nearest person month worked:	9 (funded by Cepheid)
Contribution to Project:	No change

Name:	Jennie Hermansson
Project Role:	Research Scientist, Cepheid
Nearest person month worked:	11
Contribution to Project:	No change

Name:	Raquel Rodrigues Palla
Project Role:	Research Scientist, Cepheid
Nearest person month worked:	11
Contribution to Project:	No change

Name:	Sarah Tidström
Project Role:	Research Scientist, Cepheid
eRA Commons ID:	n/a
Nearest person month worked:	11
Contribution to Project:	No change

Name:	Cherno Sidibeh
Project Role:	Temp. Research Scientist, Cepheid
eRA Commons ID:	n/a
Nearest person month worked:	12
Contribution to Project:	Troubleshooting, data analysis, biostatistical analysis

Name:	Jonathan Siegrist
Project Role:	VP Innovation, Cepheid
Nearest person month worked:	1 (funded by Cepheid)
Contribution to Project:	No change

Name:	Purvesh Khatri
Project Role:	Stanford Site PI
Nearest person month worked:	3
Contribution to Project:	No change

Name:	Michele Donato
Project Role:	Postdoc, Stanford
Nearest person month worked:	3
Contribution to Project:	No change

Name:	Mike Seda
Project Role:	PhD student, Stanford
eRA Commons ID:	n/a
Nearest person month worked:	1
Contribution to Project:	Data collection

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: 7
Contribution to Project: No change

Name: Ahla Ko
Project Role: Graduate Student, University of Arkansas
Nearest person month worked: 12
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: 3
Contribution to Project: No change

Name: Nelly Ciobanu
Project Role: Laboratory Coordinator, Moldova
Nearest person month worked: 3
Contribution to Project: No change

Name: Eugenia Cula
Project Role: Disease Investigator, Moldova
Nearest person month worked: 2
Contribution to Project: No change

Name: Alexandru Codreanu
Project Role: Laboratory Assistant, Moldova
Nearest person month worked: 2
Contribution to Project: No change

Name: Nadejda Turcan
Project Role: Laboratory Assistant, Moldova
Nearest person month worked: 2
Contribution to Project: No change

Name: Liudmila Tirsina
Project Role: Disease Investigator, Moldova
Nearest person month worked: 3
Contribution to Project: Subject enrollment

Name: Tatiana Ixari

Project Role:	Laboratory Assistant, Moldova
Nearest person month worked:	2
Contribution to Project:	Process laboratory samples

Name:	Maria Gasco
Project Role:	Laboratory Assistant, Moldova
Nearest person month worked:	2
Contribution to Project:	Process laboratory samples

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

Updates to Other Support entries for Project PI Dr. Catanzaro and UCSD Co-Investigator Dr. Rodwell are provided below. Per the instructions for this section, only categorical changes (e.g. from Pending to Current, or Current to Completed) since the last provision of Other Support information (April 2018) have been provided, and are noted in *red/italics.*

CATANZARO, ANTONINO (Project PI)

CURRENT

W81XWH1810253 Catanzaro (PI) 09/30/2018 – 09/29/2021 21% effort
Department of Defense
A Rapid Blood Test to Differentiate Latent Tuberculosis from Active Disease
We propose to develop a finger-stick triage test using non-stimulated blood that meets or exceeds WHO Target Product Profiles for a rapid biomarker-based non-sputum-based triage test for detecting TB; to extend the resulting finger-stick test to include biomarkers of pre-clinical TB.
Role: Principal Investigator
Funding agency contact: AnnMarie Gersch, PhD, Science Officer;
Previously PENDING

PA-18-484 Catanzaro/Rodwell (Multiple PI) 07/03/2019 – 06/30/2024 10% effort
NIH/NIAID
An Antigen-Detection Blood Test for Pulmonary Tuberculosis
The objective of our proposed study is to continue to improve the accuracy and usability of our prototype NanoDisk-MS assay for the detection of tuberculosis (TB) in blood samples.
Role: Multiple Principal Investigator
Funding agency contact: Caleb Waller, MBA, GMS

Previously PENDING

COMPLETED

R01AI111435 Rodwell (PI) 07/01/2014-06/30/2020
NIH/NIAID
An Integrated Tabletop Platform for Rapid Detection of XDR-TB in Clinical Samples
The goal of this project is to develop and field test a novel table-top device for rapid clinIcal diagnosis of XDR-TB in collaboration with industry partner Akonni Bisystems and the Center for Health Studies, Chisinau, Moldova.
Role: Co-Investigator

Funding Agency Contact: Elizabeth Sihombing, GMS
Previously ACTIVE

RODWELL, TIMOTHY (UCSD Co-Investigator)

CURRENT

PR171076 Catanzaro (PI) 09/30/2018 – 09/29/2021 5% effort
Department of Defense
A Rapid Blood Test to Differentiate Latent Tuberculosis from Active Disease
We propose to develop a finger-stick triage test using non-stimulated blood that meets or exceeds WHO Target Product Profiles for a rapid biomarker-based non-sputum-based triage test for detecting TB; to extend the resulting finger-stick test to include biomarkers of pre-clinical TB.
Role: Co-Investigator
Funding agency contact: AnnMarie Gersch, PhD, Science Officer; annmarie.gersch.ctr@mail.mil
Previously PENDING

PA-18-484 Catanzaro/Rodwell (Multiple PI) 07/03/2019 – 06/30/2024 20% effort
NIH/NIAID
An Antigen-Detection Blood Test for Pulmonary Tuberculosis
The objective of our proposed study is to continue to improve the accuracy and usability of our prototype
NanoDisk-MS assay for the detection of tuberculosis (TB) in blood samples.
Role: Multiple Principal Investigator
Funding agency contact: Caleb Waller, MBA, GMS

Previously PENDING

COMPLETED

R01AI111435 Rodwell (PI) 07/01/2014-06/30/2020
NIH/NIAID
An Integrated Tabletop Platform for Rapid Detection of XDR-TB in Clinical Samples
The goal of this project is to develop and field test a novel table-top device for rapid clinIcal diagnosis of XDR-TB in collaboration with industry partner Akonni Bisystems and the Center for Health Studies, Chisinau, Moldova.
Role: Principal Investigator
Funding Agency Contact: Elizabeth Sihombing, GMS
Previously ACTIVE

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

8. SPECIAL REPORTING REQUIREMENTS

Award Chart (Page 17) and Quad Chart (Page 18)

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, 2021

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 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 from 1,000 individuals

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

Funding Obtained: $3,570,150

A Rapid Blood Test to Differentiate Latent Tuberculosis from Active Disease

PR171076

W81XWH1810253

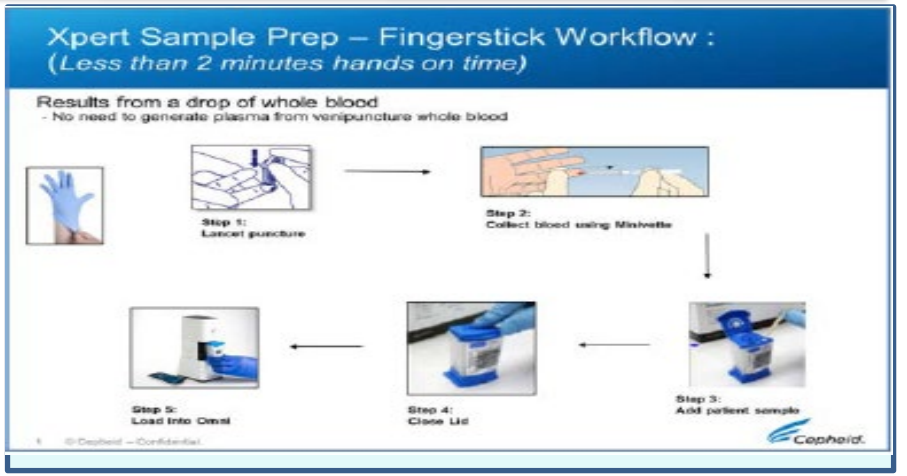
PI: Antonino Catanzaro, MD **Org:** The Regents of the University of California, San Diego **Award Amount:** $3,570,150

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 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 the Republic of Moldova. 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 Aim 2 participant enrollment; 9-gene NanoString code sets manufactured; development of antigen-stimulated signature and progress toward two prototype cartridges; establishment of UCSD OneDrive to centralize data collaboration.

Activities CY	18	19	20	21
1. Discovery & Augmentation of 3-gene TB signature				
2. Validation				
3. Enrollment & blood collection in Moldova				
4. Prototype cartridge development				
5. Field Trial				
Estimated Budget ($K)	56,410	376,361	1,364,910	1,772,469

Updated: 10/29/2020

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 Goal – Field Trial

☐ Finalize prototype cartridge; field test in Moldova

Comments/Challenges/Issues/Concerns: Subaward liens as of 9/30/20 = $714,521, not included in Actual Expenditure amount below

Budget Expenditure to Date

Projected Expenditure: $2,268,008

Actual Expenditure: $1,206,858