

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, CA

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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 novel 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 validate the signatures and completed internal quality testing toward finalizing the prototype cartridges for field evaluation. The new field site in Pakistan was established, and during the No Cost Extension (NCE) period prospective enrollment began for 3-gene and antigen-stimulated cartridge field testing. Completion of enrollment and data analysis will take place during the additional one-year NCE period.					
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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 at risk 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, Pakistan, WHO, TPP, biomarkers.

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 ($\geq 90\%$ sensitivity when compared with the confirmatory test for active TB (both pulmonary and extrapulmonary) and $\geq 70\%$ 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 clinical patients in Pakistan who are at risk 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 prototype cartridges using blood, finalize prototype cartridge for field testing

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:

- a) *Initiation of participant enrollment for Aim 3:* In Q3 of Year 4, we began participant enrollment at Federal General Hospital (FGH) for Aim 3 goals. Additionally, we started

working with a second site in Pakistan, the Rawalpindi Leprosy Hospital (RLH) to help increase study recruitment. Activities completed during Year 4 that preceded this milestone included finalizing the detailed study protocol, supplies list, and laboratory workflows, conducting protocol and data collection training with the site staff, and finalizing the site's subaward. DoD IRB approval was attained early in Year 4, and annual continuing reviews for UCSD and the local IRB in Pakistan were conducted. To date, 352 participants have been enrolled, 191 from FGH and 161 from RLH.

The UCSD coordinating center maintained weekly virtual meetings with site staff beginning early in Year 4 to implement study procedures, which allowed for establishment of a close working relationship between team members from the coordinating center and field site. Also, during Year 4, the University of Arkansas data core finalized the data collection forms and programmed them in REDCap, the electronic data capture system. Since initiation of enrollment, we have monitored data collection regularly and conducted routine quality checks.

- b) *Field testing of Xpert TB Host Response (XHR) cartridges initiated:* Field testing of the 3-gene Xpert Host Response (XHR) began during Year 4. Enrollment and real-time cartridge testing began in May 2022, and for initial tests runs, daily exports of raw data from samples processed with XHR cartridges and Xpert MTB-Rif Ultra cartridges were reviewed by Cepheid staff with expertise to do quality checks. One minor issue that was identified was quickly resolved, and since meeting all quality standards, Pakistan team members now upload data on a weekly basis for routine monitoring by Cepheid. As previously reported, while the Latent TB cartridge was being finalized, blood was simultaneously collected in antigen-stimulated tubes and frozen for testing at a later date (see next section).
- c) *Prototype Latent TB cartridge completion and delivery:* During Year 4, Cepheid completed development of the prototype Xpert Latent TB (XLTB) cartridge for field testing. To reach this milestone several activities occurred: first, Cepheid laboratory staff completed oligo screening and selection, which resulted in achievement of robust multiplex-amplification of all 10 RNA targets. During Q2, staff evaluated oligo design modifications, selected final oligo designs, ordered, received, quantitated, and qualified oligo lots for the prototype assay. Cepheid also performed key component range finding and optimization experiments and locked reagent formulations. During Q3, Cepheid completed development of the cartridge, finalized dry and wet reagents for the XLTB assay, completed the assay definition file (sample preparation, thermocycling protocol, and analysis settings) and the uploaded assay definition file to OneDrive for field site access. The manufactured prototype assays created on Cepheid's automated ROBAL lines were shipped to the field site. As previously reported, the shipment was sent in Q3, but significant delays with customs in Pakistan delayed receipt of the shipment by the field site. The shipment arrived only in late Q4, but still in good condition and Cepheid determined that the reagents still met internal QA/QC standards. While awaiting the delivery, Cepheid performed remote training with the Pakistan staff (July 2022). The accelerated kit stability study was also completed during Q4 as planned.

Concurrently during Q4, Cepheid tested the final cohort enrolled in Moldova of 75 antigen-stimulated PAXgene-preserved blood samples (25 each of active TB, latent TB, and non-TB infected) which were collected to de-risk the Aim 3 study in Pakistan by validating the specific sample stimulation peptides planned for use in the prototype cartridge based on antigen-stimulated blood. Comparison of three antigen tube types (TB1 from Qiagen, 2-antigen from Lionex, and 3-antigen from Lionex) was also completed. The 2-antigen tube

from Lionex was ultimately selected by Cepheid and sent to Pakistan for the study. The comparison study enabled Cepheid to assess whether future adjustments for improvement would be advantageous.

d) *Manuscript development for work on in vitro performance testing of novel 9-gene signature:* During Q1 of Year 4, Stanford collaborators continued analysis of data from the *in vitro* performance testing of the 9-gene signature and began developing the manuscript to publish this work. Stanford presented the analysis to UCSD during Q2 and provided a draft for co-author review during Q4. At this time the work has been accepted in poster form at the Conference for the International Union of Tuberculosis and Lung Disease, and the manuscript is undergoing final drafts before submission.

- 2) **Specific Objectives:** The major activities in Year 4 supported the specific objectives outlined in the SOW for Year 4 including: prototype cartridge development, prototype cartridge component field testing, data analysis, and scientific collaboration.
- 3) **Significant Results or Key Outcomes:** Significant results of the Year 4 work were finalization and manufacturing of the Cepheid prototype latent TB cartridge for field testing, and active real-time field testing of the 3-gene Host Response cartridge.
- 4) **Other Achievements:** Other major achievements of the Year 4 work were initiation of enrollment and data collection, and successful implementation of QuantiFERON (for LTBI reference testing) which had not previously been used in the Pakistan NTRL. As this was a new field site, many administrative and programmatic achievements were necessary to ensure enrollment and laboratory testing were successfully implemented. The field site maintains a high level of performance.
- 5) **Stated Goals Not Met:** The main goal in the SOW that was not met in Year 4 was to complete planned enrollment and field testing of the prototype XLTB cartridge. At the time of this report, we have been granted an additional 1-year no cost extension to complete the work. This is discussed in greater detail in subsequent sections.

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

Nothing to report.

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 we plan to continue enrollment at the field site in Pakistan and provide ongoing field study and data collection support. We will continue testing the XHR cartridges in real-time while also starting to test both frozen samples and fresh samples (in real time) using the XLTB cartridges. We anticipate that we will complete patient enrollment and laboratory testing for Aim 3 during the upcoming project year and then will collate Xpert cartridge data with clinical data to complete field-testing performance analysis on both cartridges. Data will be used to train and test final algorithms and determine assay performance. Results will be shared in the final project report and published in one or more peer-reviewed journals. We also plan to submit the near final draft manuscript in collaboration with the Stanford study team with results of the 9-gene signature analysis.

4. IMPACT:

This study will provide additional important evaluation data for the existing **XHR** cartridge to support the completion of development and commercialization of this product. But most importantly, our study will provide unique and novel preliminary data for the Cepheid antigen-stimulated latent TB cartridge. If the performance of this prototype meets performance expectations, this product will be capable of detecting both LTBI and differentiating it from active TB, which is currently not possible with existing TB assays. This is the first large clinical study of the **XLTB** assay and prototype product to determine if the early positive laboratory results can be reproduced in the field. If confirmed, Cepheid could begin final development of this novel product that has the potential to transform how latent TB infection and active TB are diagnosed at a global population scale.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

We anticipate that initiation of QuantiFERON for LTBI diagnosis during this study (the test was not previously implemented in the Pakistan NTRL) could lead to adoption of latent TB testing more routinely in this region. Preliminary results of this testing within the study population indicate that approximately 45% of participants have latent TB infection – which is the first broad, TB risk-based estimate of LTBI prevalence in Pakistan. If this testing were to be expanded in the population at risk for TB, the impact on public health would be significant.

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

During Year 4 the most significant problems encountered were related to timely delivery of study supplies shipped from Cepheid in Sweden. Two shipments were sent from Sweden to Pakistan during this timeframe, and both had significant delays due to customs clearance obstacles within Pakistan resulting from political instability and local governmental changes, which resulted in slower than expected approvals. The first supply delay caused a delay in initiation of participant enrollment, and the second delay resulted in a required pause in enrollment at n=187 due to depletion of the antigen-stimulated collection tubes required for collection of blood for the XLTB prototype cartridge. The second delayed shipment included the XLTB cartridges for field testing, in addition to a replenishment of supplies for collection of samples for both the XHR and XLTB cartridges. The UCSD coordinating center inquired about potential workarounds to avoid a pause in enrollment, but Cepheid advised against this as there was not a suitable alternative for the antigen-stimulated tube approved for the research. Had we continued to collect patient blood samples for processing in the XLTB cartridges, we would have had to freeze the samples before they were stimulated, which is not compatible with the functioning of the XLTB cartridge. However, the shipment was ultimately delivered to the NTRL at the end of Q4. Though after the current reporting period, as of the time of this report submission, participant enrollment has re-started and frozen and fresh protocols for the XLTB cartridge testing are continuing.

The delays in enrollment and testing also mean that we can no longer achieve the total sample size of N=1,000 that we originally proposed. Based on the additional samples collected in Aim 2, and the expected performance of the XHR and XLTB cartridges, we evaluated the potential effects of reducing the total sample size for the Aim 3 study to N=500. We determined that we could still meet the project goals with acceptable precision with N=500. Pakistan has now received all the supplies needed to complete the revised enrollment goals and therefore we do not currently plan to attempt an additional shipment from Cepheid to Pakistan as the ongoing customs delays would likely push delivery of supplies beyond the NCE period.

Changes that had a significant impact on expenditures

Delays in the start of participant enrollment in Pakistan resulted in unexpended funds as we concluded our first NCE period. The approval of a 2nd NCE period, extending the project through September 2023, will allow us to meet the proposed goals and deadlines described in our NCE request.

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

- Gupta S, Rao AM, Scott M, Crudu V, Rodwell TC, Catanzaro D, Catanzaro A, Khatri P. A Nine-Gene Blood-Based Signature Meets the World Health Organization Target Product Profiles for Diagnosis of Active Tuberculosis and Predicting Progression from Latent to Active Disease. InC106. FIRE TB LIVES ON 2022 May (pp. A4924-A4924). American Thoracic Society.

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
Project Role:	Principal Investigator, UCSD
Nearest person month worked:	1
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 (<i>funded by Cepheid during NCE</i>)
Contribution to Project:	Analytical and field studies with Xpert TB/LTBI RUO prototype assay
Name:	Antonio Ascue Avalos
Project Role:	Research Scientist, Cepheid
Nearest person month worked:	11 (<i>funded by Cepheid during NCE</i>)
Contribution to Project:	Analytical and field studies with Xpert TB/LTBI RUO prototype assay
Name:	Sarah Tidström
Project Role:	Senior Scientist, Cepheid
Nearest person month worked:	1 (<i>funded by Cepheid during NCE</i>)
Contribution to Project:	Analytical and field studies with Xpert TB/LTBI RUO prototype assay
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:	No change
Name:	Donald Catanzaro
Project Role:	University of Arkansas Site PI
Nearest person month worked:	4

Contribution to Project:	No change
Name:	Sabira Tahseen
Project Role:	Site PI, Pakistan
Nearest person month worked:	1
Contribution to Project:	Local coordination of personnel, budget, and administrative requirements for study initiation at site; participated in teleconferences to discuss project planning and scientific approach. Coordinated enrollment effort and IRB approvals; translation of consent forms.
Name:	Alamdar Hussain Rizvi
Project Role:	Site Project Manager, Pakistan
Nearest person month worked:	1
Contribution to Project:	Participated in teleconferences to discuss project planning and scientific approach; coordinated preliminary remote site training on study procedures and protocols; coordinated shipments of supplies and equipment, prepared site for enrollment initiation; procured laboratory supplies for specimen collection

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 updated Other Support information (October 2021).

What other organizations were involved as partners?

Organization Name:	<u>University of Arkansas</u>
Location of Organization:	Fayetteville, Arkansas
Partner's contribution to project:	a) Facilities (PI office space, data core facilities) b) Collaboration

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

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, 2023

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 ($\geq 90\%$ sensitivity when compared with the confirmatory test for active TB (both pulmonary and extrapulmonary) and $\geq 70\%$ 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.
- Gupta S, Rao AM, Scott M, Crudu V, Rodwell TC, Catanzaro D, Catanzaro A, Khatri P. A Nine-Gene Blood-Based Signature Meets the World Health Organization Target Product Profiles for Diagnosis of Active Tuberculosis and Predicting Progression from Latent to Active Disease. InC106. FIRE TB LIVES ON 2022 May (pp. A4924-A4924). American Thoracic Society.

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 clinical patients

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).

Xpert Sample Prep – Fingerstick Workflow : (Less than 2 minutes hands on time)

Results from a drop of whole blood
- No need to generate plasma from venipuncture whole blood

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Accomplishments: Initiation of Aim 3 enrollment in Pakistan; field testing of Xpert TB Host Response cartridges; prototype Latent TB cartridge completion and delivery to field site; manuscript development and presentation of *in vitro* testing of novel 9-gene signature.

Activities CY	18	19	20	21	22	23
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	1,131,665	426,089	321,530

Updated: 10/29/2022

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

CY21-23 Goal – Field Trial in Pakistan

- Finalize prototype cartridge
- Field test at clinical site

Budget Expenditure to Date

Projected Expenditure: \$3,570,150

Actual Expenditure: \$3,141,444

A Nine-Gene Blood-Based Signature Meets the World Health Organization Target Product Profiles for Diagnosis of Active Tuberculosis and Predicting Progression from Latent to Active Disease

S. Gupta¹, A. M. Rao¹, M. Scott¹, V. Crudu², T. C. Rodwell³, D. Catanzaro⁴, A. Catanzaro³, P. Khatri¹; ¹Department of Medicine, Stanford University, Palo Alto, CA, United States, ²Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Moldova, Republic of, ³Division of Pulmonary, Critical Care and Sleep Medicine, University of California, San Diego, La Jolla, CA, United States, ⁴Department of Biological Sciences, University of Arkansas, Fayetteville, AR, United States.

Rationale: The World Health Organization (WHO) reported that approximately 10 million people developed active tuberculosis (ATB) in 2020, of which only 5.8 million were diagnosed and 1.5 million patients died. The current reference-standards for diagnosis of ATB: sputum culture and smear microscopy, have several limitations, including low sensitivity, difficulty in obtaining sputum samples, and inability to predict progression from latent TB (LTB) to ATB, highlighting a need for non-sputum-based diagnostics. As part of its End TB strategy, the WHO has identified the need for non-sputum-based tests that meet target product profiles (TPP) of 90% sensitivity and 70% specificity for diagnosis of ATB and 75% sensitivity and specificity for predicting progression from LTB to ATB. Although several single-cohort studies have demonstrated promising blood-based gene expression signatures for diagnosis of ATB, they often fail to generalize to larger patient populations with more biological and clinical heterogeneity. However, a 3-gene blood-based signature, identified using diverse datasets, has been successfully translated into a prototype point-of-care test that meets the WHO TPPs. The successful translation of the 3-gene signature demonstrated the power of integrating large amounts of heterogeneous data to identify robust, generalizable disease signatures. We hypothesized that including more diverse datasets comprising patients with ATB or other lung and inflammatory diseases, would identify novel, highly robust host response-based signatures for diagnosis of ATB and predicting progression from LTB to ATB that meet performance expectations of the WHO TPP. **Methods:** We used a multi-cohort analysis framework to integrate and analyze data from 3,615 peripheral blood samples in 49 publicly available transcriptomic datasets (discovery cohorts), from healthy controls and patients with LTB, ATB, and other diseases (COPD, viral infections, sarcoidosis, lung cancer, etc.). We then used (1) 3,836 blood samples from 28 retrospective datasets and (2) 334 prospectively collected blood samples from Moldova as validation cohorts. **Results:** Using the discovery cohorts we identified a 9-gene signature, which classified patients with ATB with 90.1% sensitivity and 81.7% specificity in retrospective validation cohorts and with 90.2% sensitivity and 68.9% specificity in the prospective Moldova cohort (Figure 1A-B). In a longitudinal cohort of adolescents, the 9-gene signature predicted progression from LTB to ATB up to 1 year prior to sputum conversion with 76% sensitivity and 83.3% specificity (Figure 1C). Finally, it also correlated with lung inflammation in the Catalysis Treatment Response Cohort (Figure 1D). **Conclusion:** Overall, the 9-gene signature meets the WHO TPPs required for the End TB strategy.

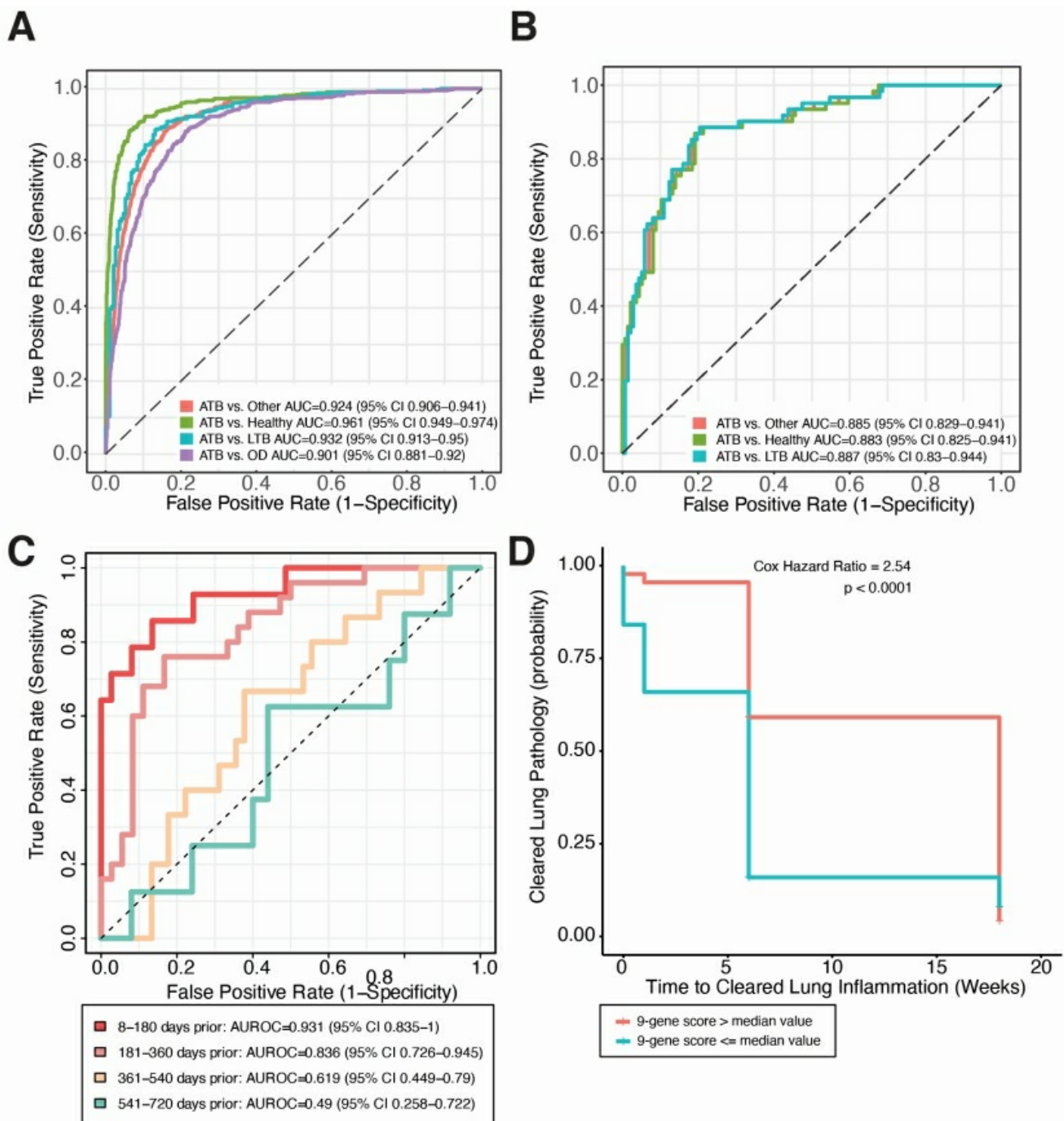


Figure 1. 9-gene signature validates in independent retrospective and prospective cohorts.

(A) ROC curves for comparing ATB vs. all other samples (Other), or individually comparing ATB vs. Healthy, LTB or Other Disease (OD) samples, if present, in independent validation and (B) a prospective Moldova cohort. (C) ROC curves comparing progressor and non-progressor samples collected at different time points in the ACS cohort. (D) A Cox regression analysis shows that patients with a 9-gene score higher than the median value have a significantly higher probability of cleared lung pathology across all time points.

All ROC curves are obtained with The 9 Gene score, which is computed as the difference of geometric means of the upregulated and downregulated genes.

TB, tuberculosis; ATB, active tuberculosis; LTB, latent tuberculosis; ROC, receiver operating curve; AUROC, area under the receiver operating curve; ACS, Adolescent cohort study.

This abstract is funded by: Bill & Melinda Gates Foundation OPP1113682; DoD USAMRAA Award W81XWH-18-1-0253