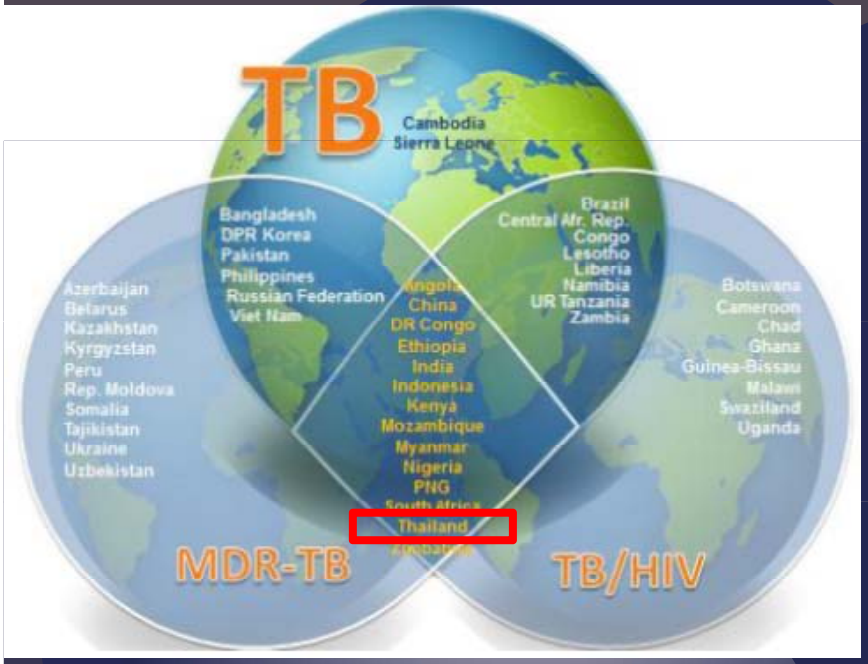
Mining through Gene Expression Profiles for Novel Biomarkers for Tuberculosis

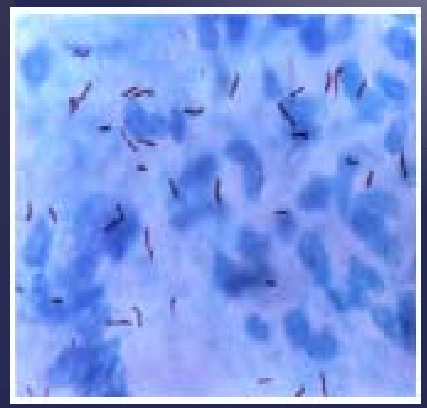
Nusara Satproedprai Ph.D.
Medical Genetics Center, Medical Life Science Institute
Department of Medical Sciences
Ministry of Public Health
Thailand

Outline

- **Background of Tuberculosis**
- **Novel Host Gene expression biomarker for TB diagnosis**
  - Discovery
  - New Diagnostic tool
  - New method for Treatment Monitoring
  - For stratification of Latent Tuberculosis

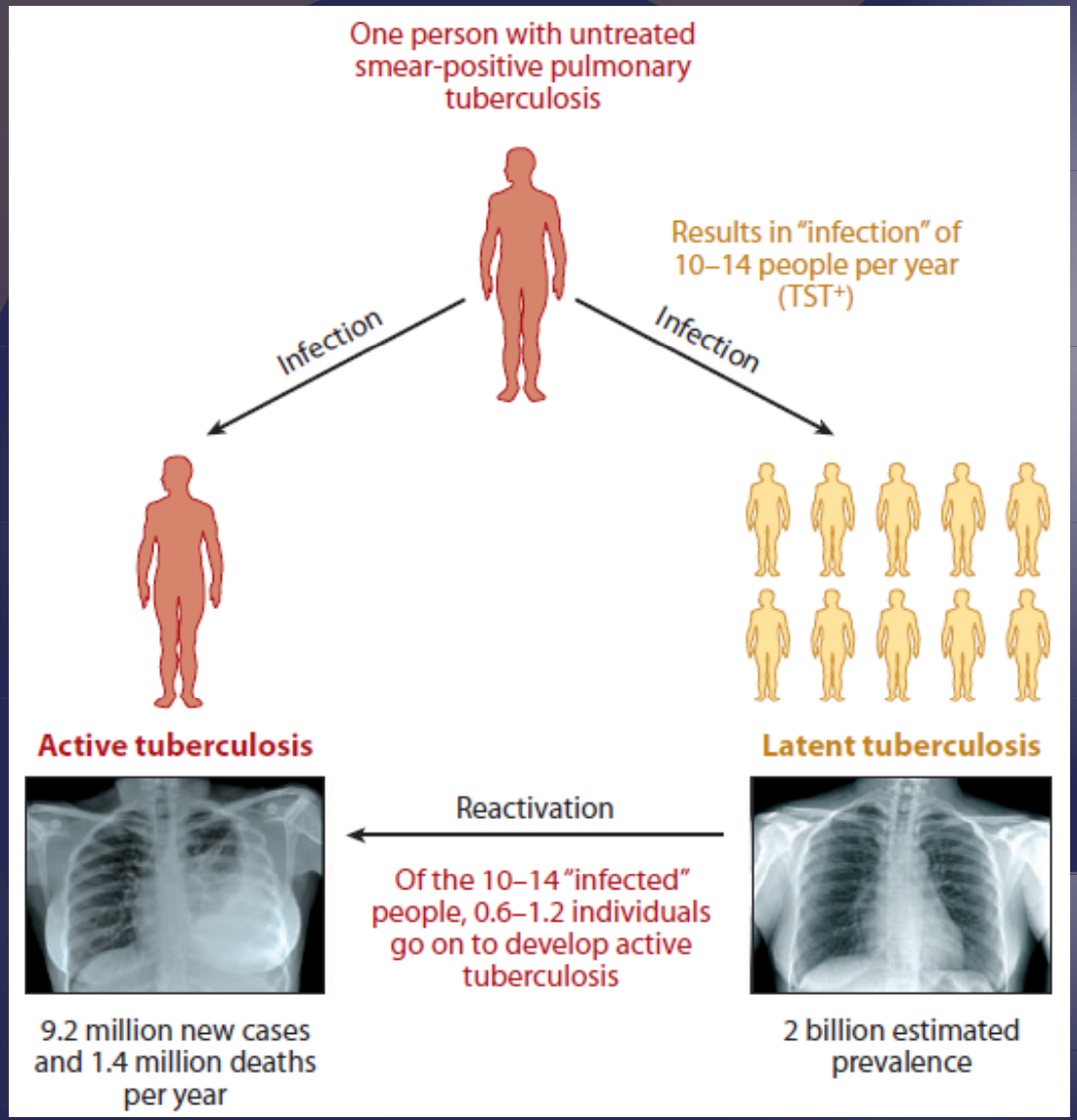
Problems





- WHO Global Tuberculosis Report 2016: Total Tuberculosis case 10.4 M, Dead 1.8 M, only 61% of those cases were diagnosed
- Thailand is one of the 30 TB high burden country; incidence 119,000 new TB cases, only 60% were bacteriologically confirmed
- Current TB diagnostic varied in sensitivity and usually takes long turn around time (2 weeks – 2months) and rely much on the quality of the sputum samples
- Low bacterial load in HIV infected individual and children infected with TB and often seen disseminated TB rather than pulmonary TB

Background : TB Infection



Active TB disease (ATB):

- is characterized by systemic features i.e. fever, weight-loss with localized symptoms of tissue destruction

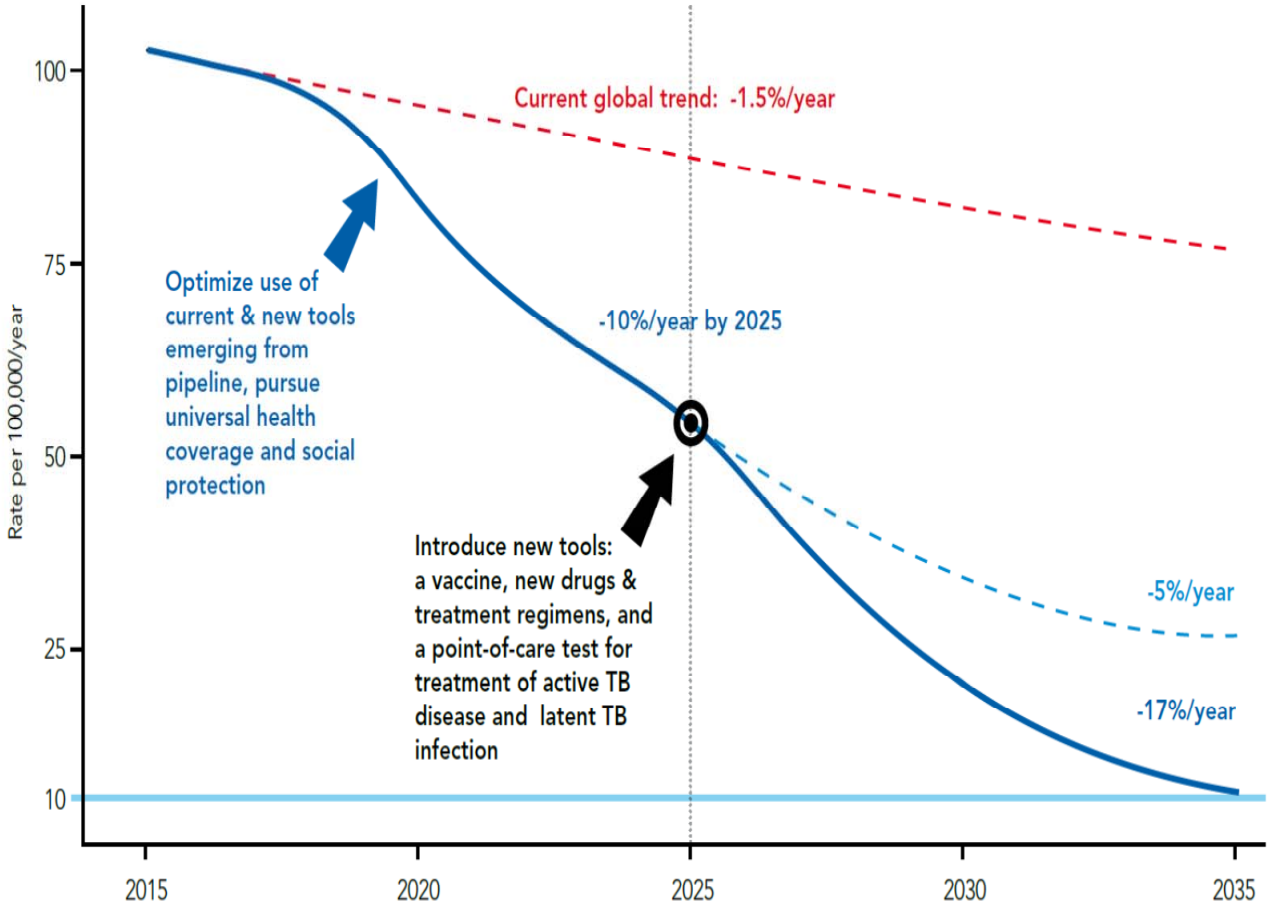
Latent TB Infection (LTBI):

- Infections with presented immune reactions but no symptom

Pascual V. et al. Annu. Rev. Immunol. 2010.

Ways to Tackle TB Problems

Figure 2. Projected acceleration in the decline of global tuberculosis incidence rates to target levels

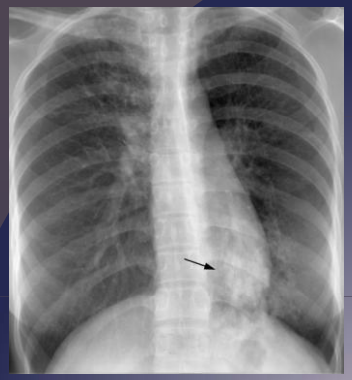


The End TB strategy, WHO 2015

New Tools

- Vaccine
- Treatment
- Diagnostics
  - Early Detection
  - Rapid and more accurate
  - POCT for active TB and latent TB
  - Better biological indicators for safety and efficacy
  - Biological indicators for treatment response

Diagnosis of TB

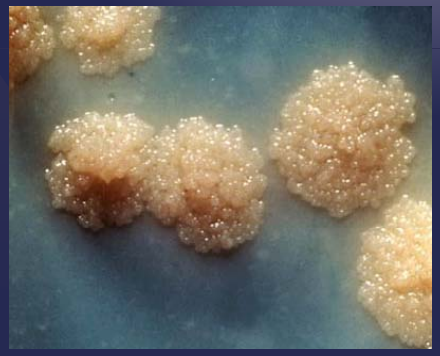


Clinical Diagnosis

Molecular Diagnostics

Acid fast bacilli staining of Sputum smear

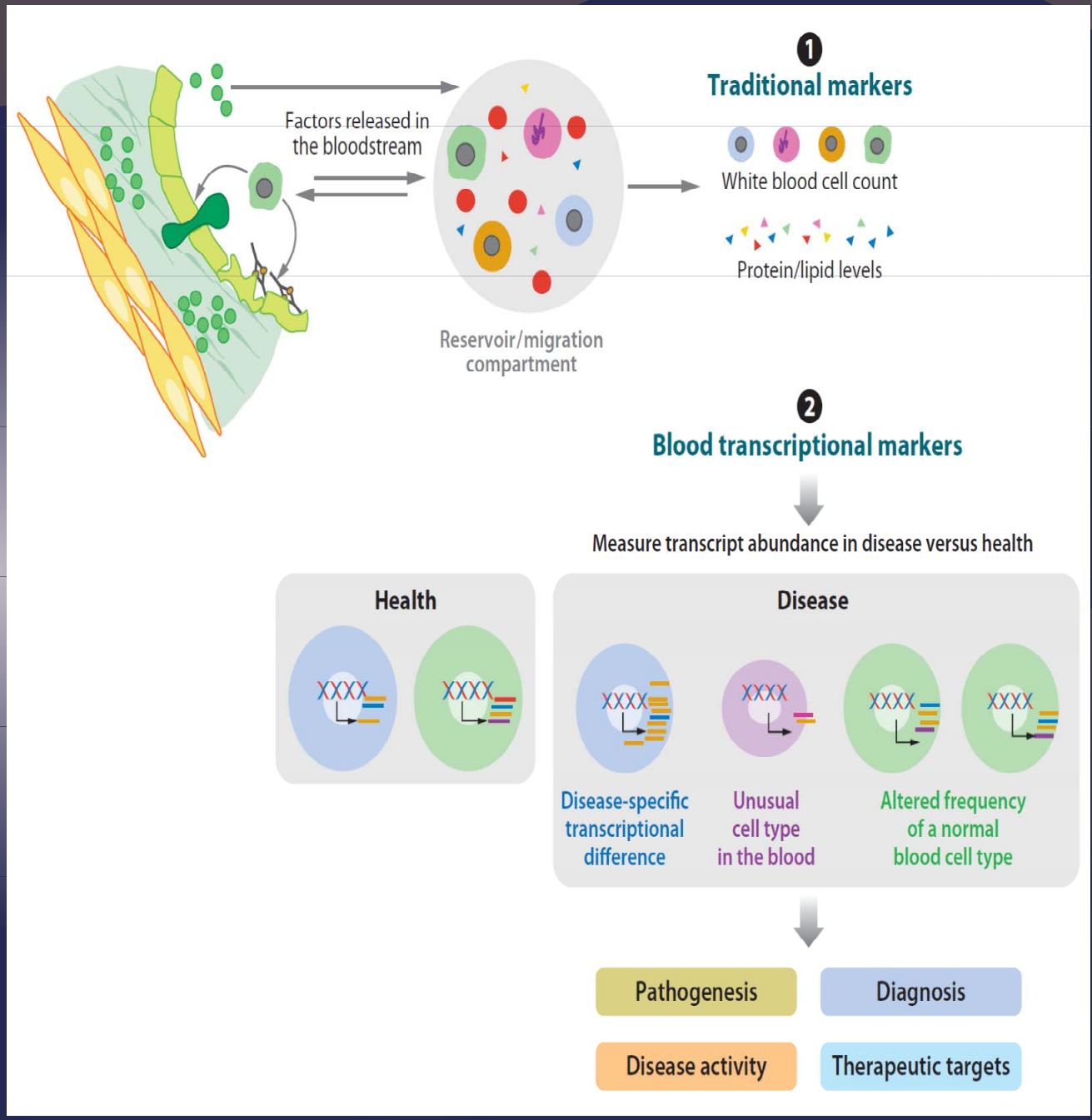
Interferon Gamma release assay (IGRA)



TB Culture

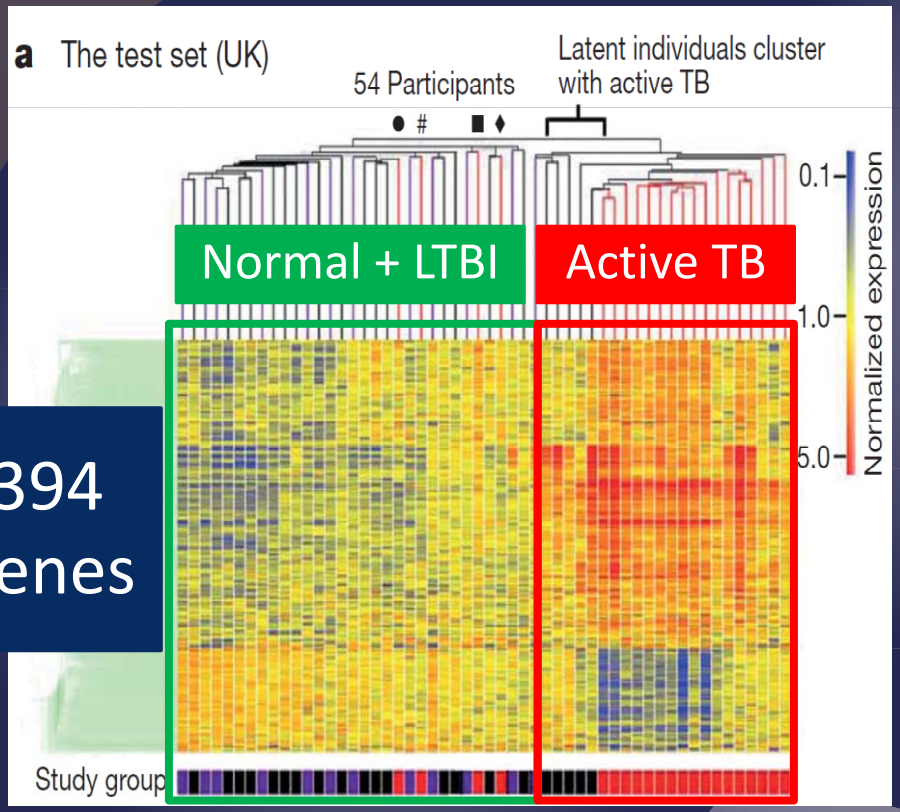
Rapid Test

Blood gene expression analysis delivers a snapshot of global host responses against infection

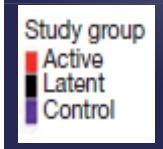


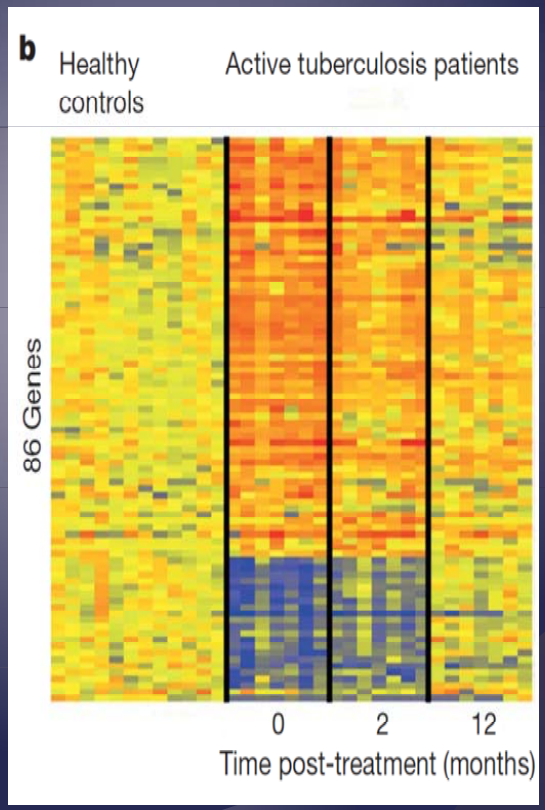
Pascual V. et al. Annu. Rev. Immunol. 2010.

Transcriptome Analysis Identified "Blood Transcriptional Signatures" Exclusive for TB



394 genes



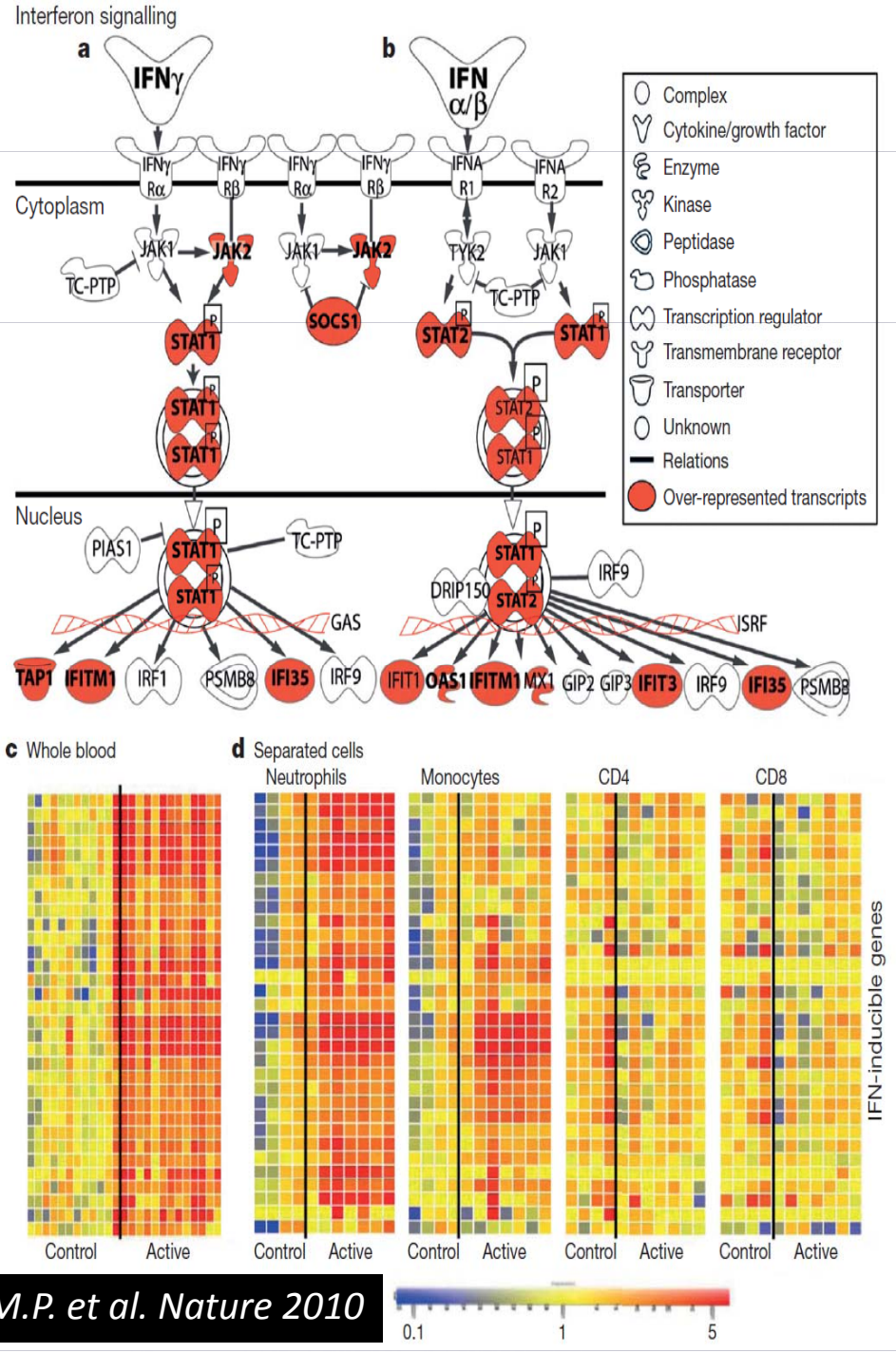


- Identified genes that differentially expressed only in Active TB but not LTBI or healthy
- Global gene expression pattern from most of LTBI cases resembles that of healthy
- "Active TB Pattern" changed back to "Healthy Pattern" after treatment completion
- Most of the highly differentiated genes were belonged to IFN-responsive genes family

Berry M.P. et al. Nature 2010

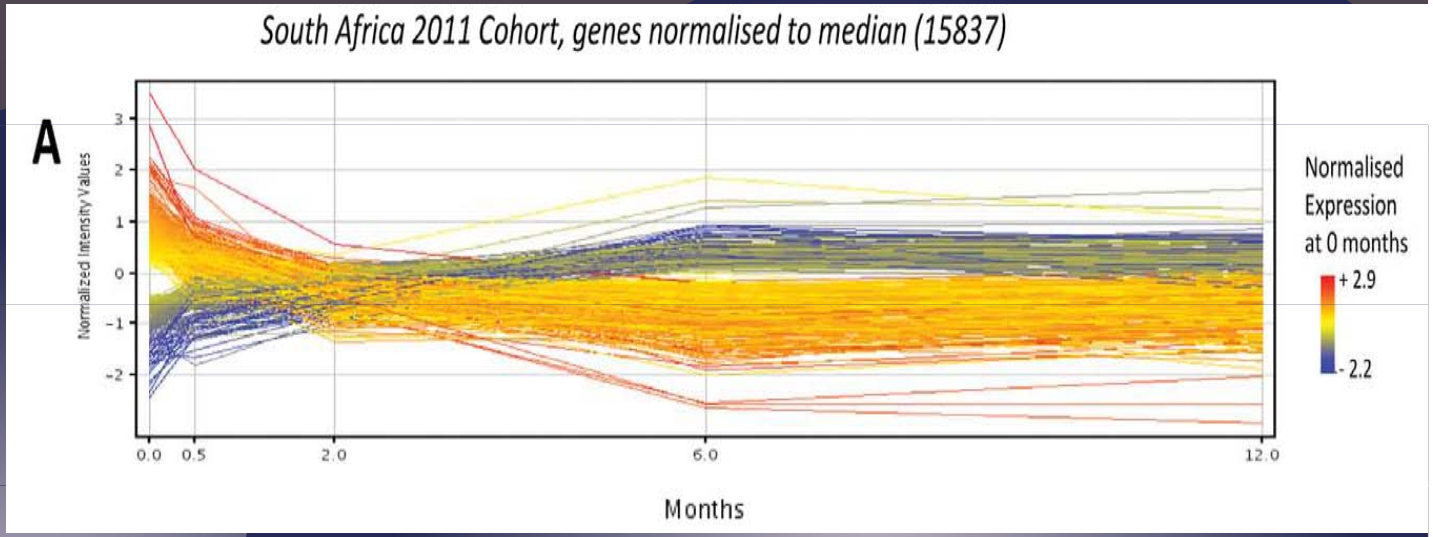
Modular and pathway analysis revealed that the TB signature was dominated by a neutrophil-driven interferon (IFN)-inducible gene profile

- Pathway analysis revealed that most of the highly differentiated genes among active TB and healthy control are from IFN gamma and Type I Interferon pathways
- Modular analysis from various types of cells isolated from the same whole blood samples shows that induced IFN genes are overexpressed in Neutrophils and Monocytes but not CD4- and CD 8 - T cells

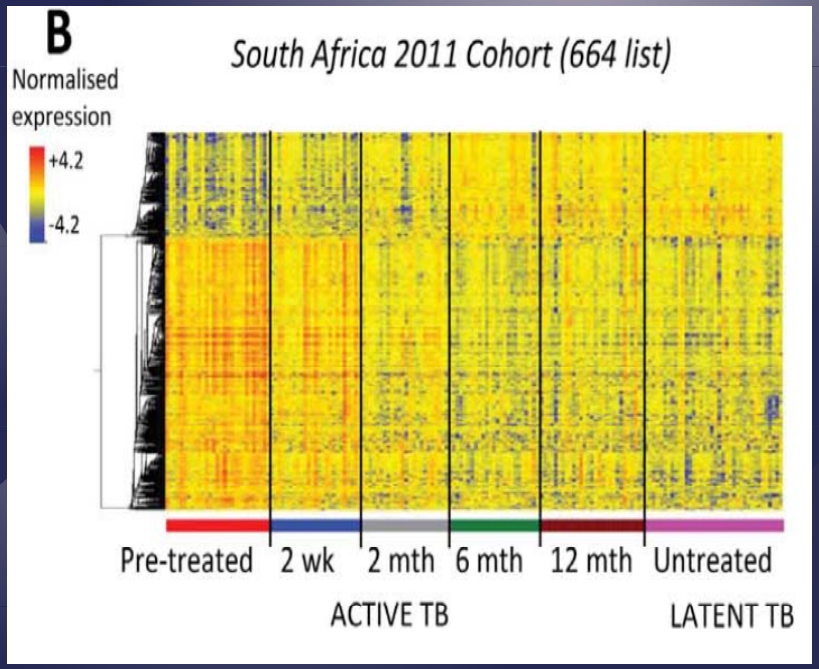


Berry M.P. et al. Nature 2010

Changes in Genes Expressions were Detected as Early as 2 Weeks after anti-TB Treatment Initiation

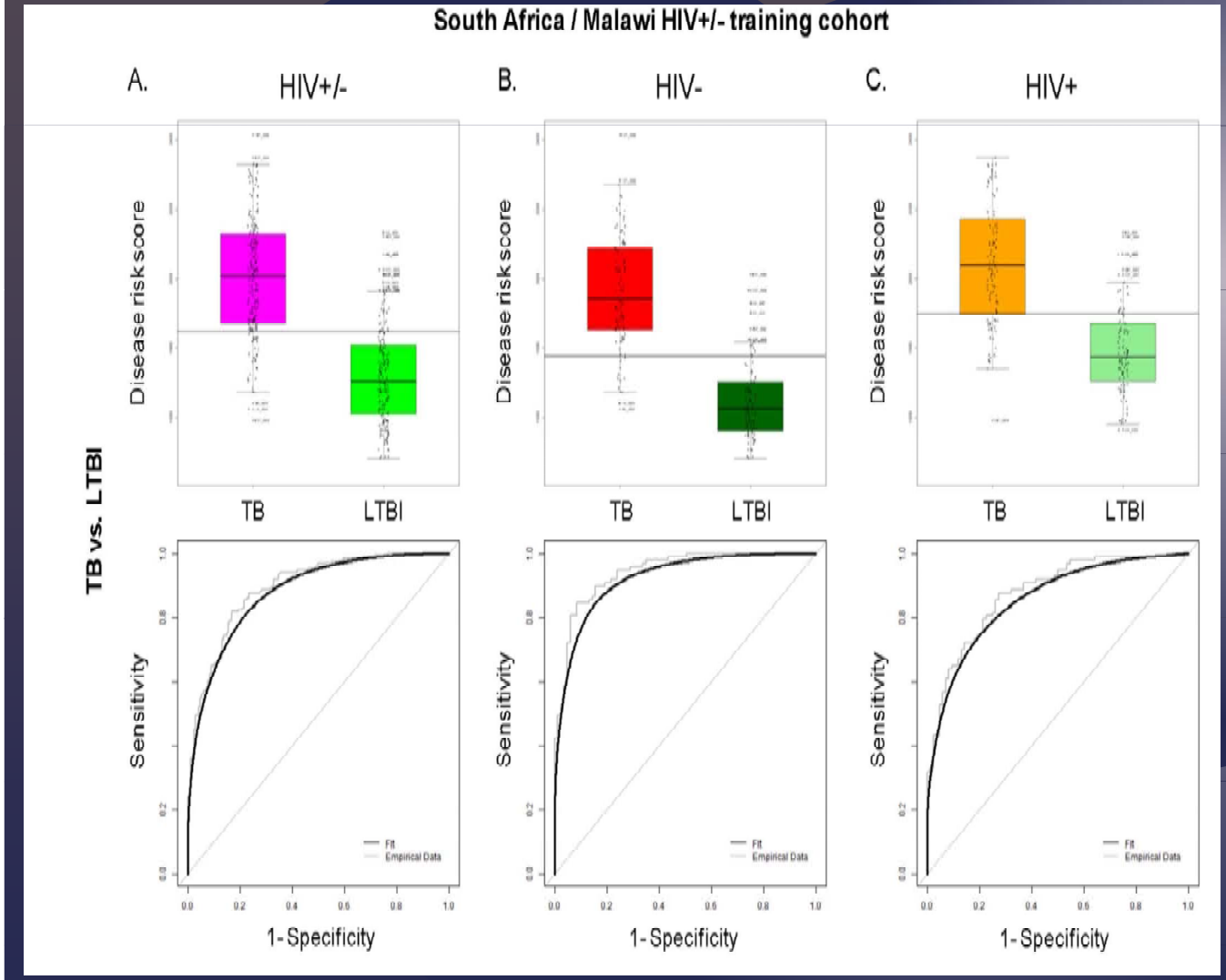


- Gene expression profiles in blood of African untreated TB patients were compared with profiles after 2 wks, 2 months 6 months and 12 months after initiation of anti-TB treatment
- Changes were seen as early as 2 wks and gradually reduced to normal
- Pathway analysis reveals genes with highest differentiation are from IFN signaling pathway



Bloom, C. I et al. 2012 PLoS ONE, 7(10).

Blood gene expression signatures can differentiate Active TB in an HIV-TB co-infection cohort



- 27 minimum transcripts were identified to differentiate active TB patients from latent TB individuals
- Develop a disease risk score that takes expression level of the 27 genes to calculate the risk of having active TB
- Disease risk score can be used for diagnosis of active TB with 95% sensitivity and 90% specificity
- Differentiation power does not decrease from HIV status

Kaforou, M., et al. PLoS Med 2013, 10(10)

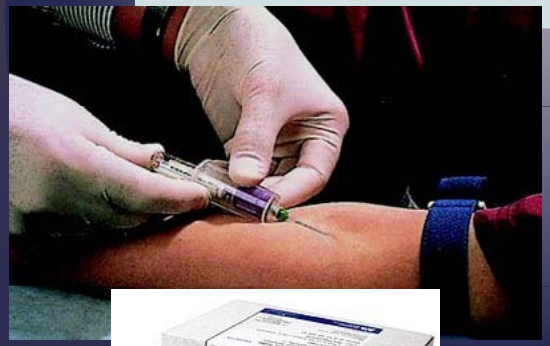
New Diagnostic Method for TB

- ✓ For use with smear negative TB, HIV-TB and Childhood TB
- ✓ Use samples other than sputum
- ✓ Novel Biomarkers
- ✓ Faster turnaround time
- ✓ High sensitivity and specificity

Gene Expression biomarkers in Blood

Methods

1. Blood Collection



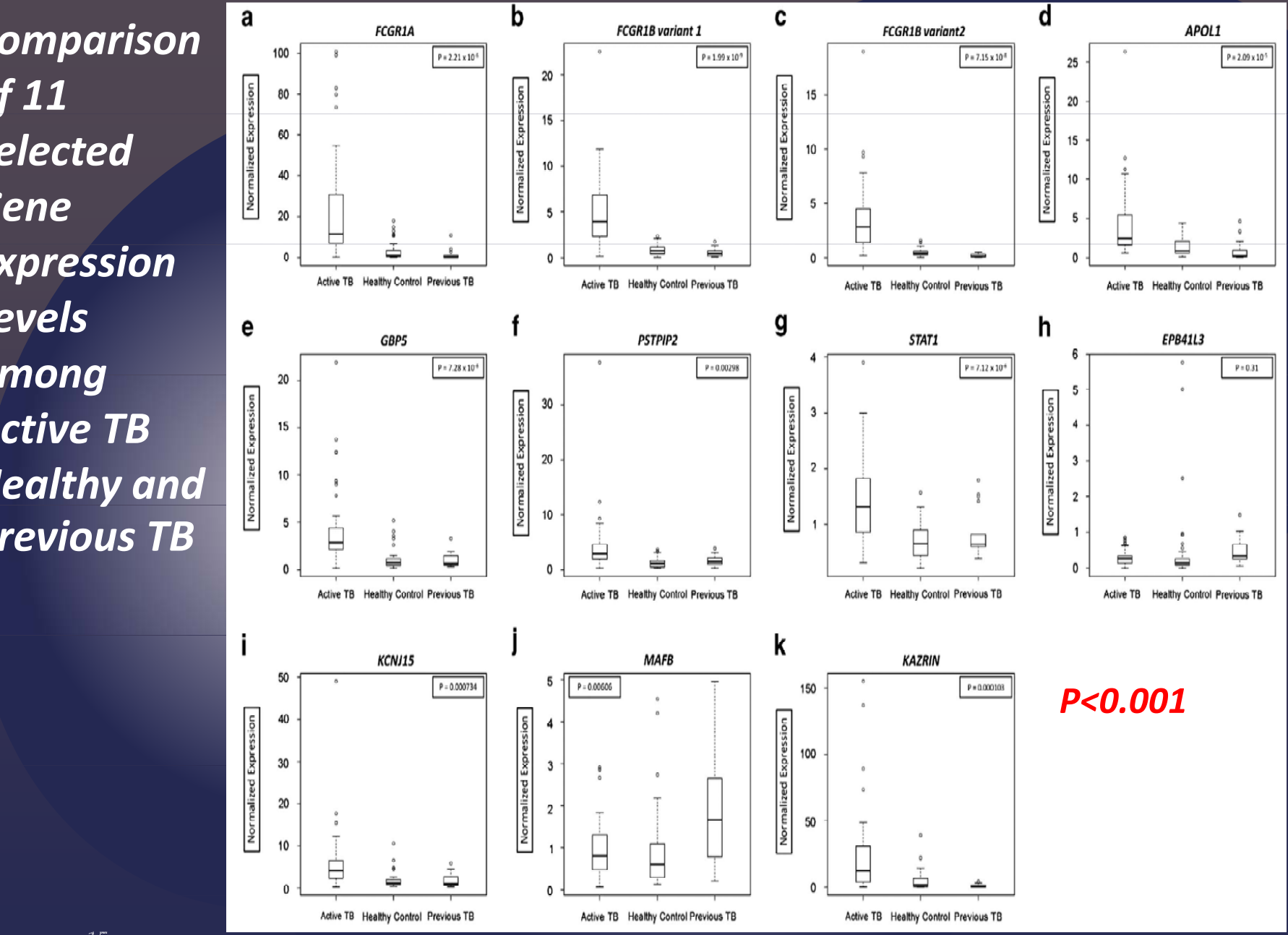
2. RNA Extraction and Reverse Transcription

3. Measure level of target RNA (Expression)

Methods

1. Selected genes with highest differentiate power from public TB microarray data
2. Tested the “Top 20” genes in small sample size (10 TB and 10 Healthy)
3. Defined a combination of genes that best predict active TB → “TB Sick Score”
4. Tested in with bigger sample size (38 TB, 40 Healthy, 18 Treated TB)

Comparison of 11 Selected Gene Expression Levels among Active TB Healthy and Previous TB



P<0.001

Satproedprai N. et al. 2015 genes and immunity

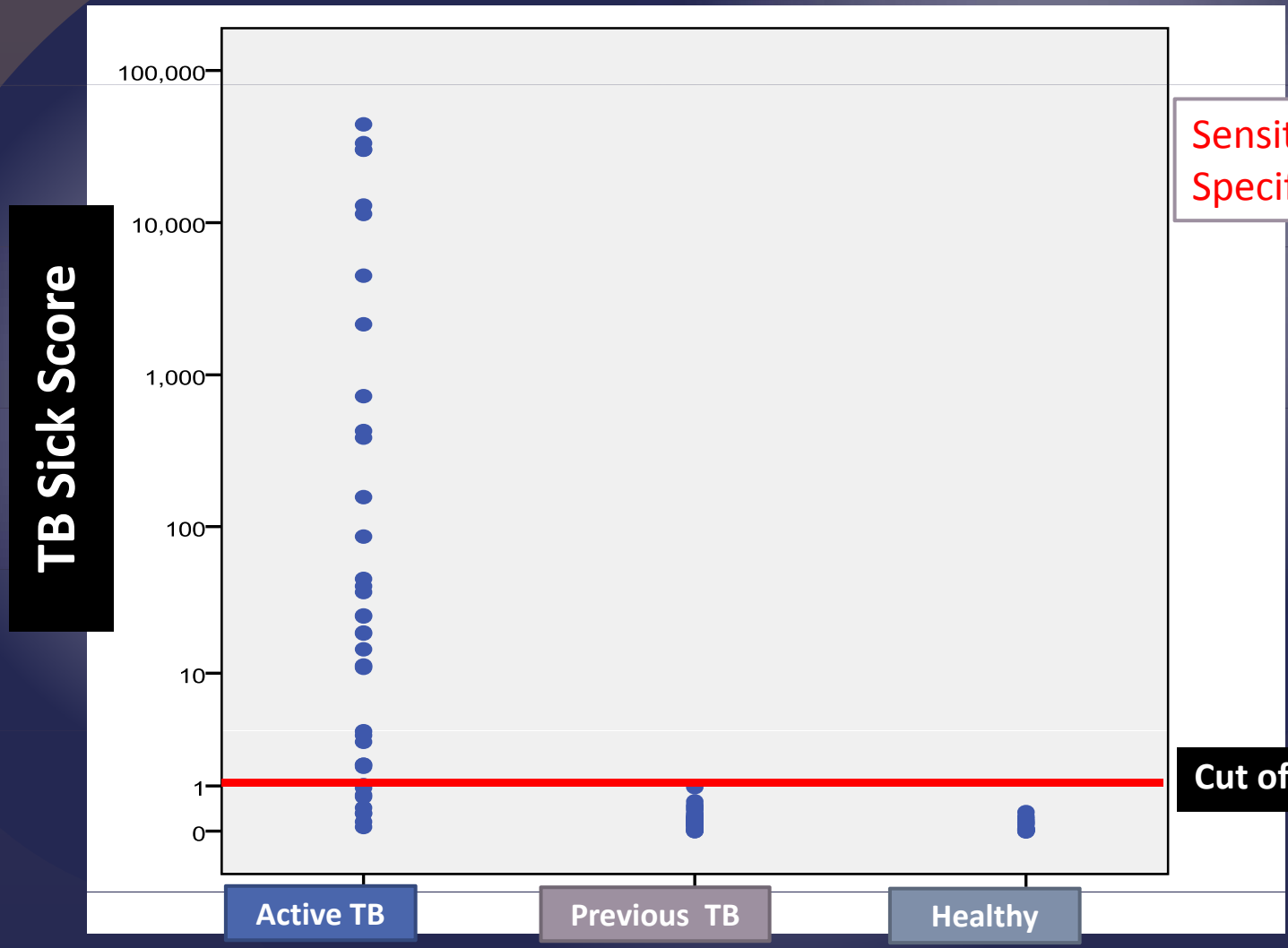
List of Genes in the Panel

FCGR1A	*FCGR1B1*
FCGR1B2	*APOL1*
STAT1	*KAZN*
*HPRT1***	*MAFB*

Selection suggested by Genome-wide association study

** Internal control gene

Results: "TB Sick Score" is highly specific for Active TB patients



Satproedprai N. et al. 2015 genes and immunity

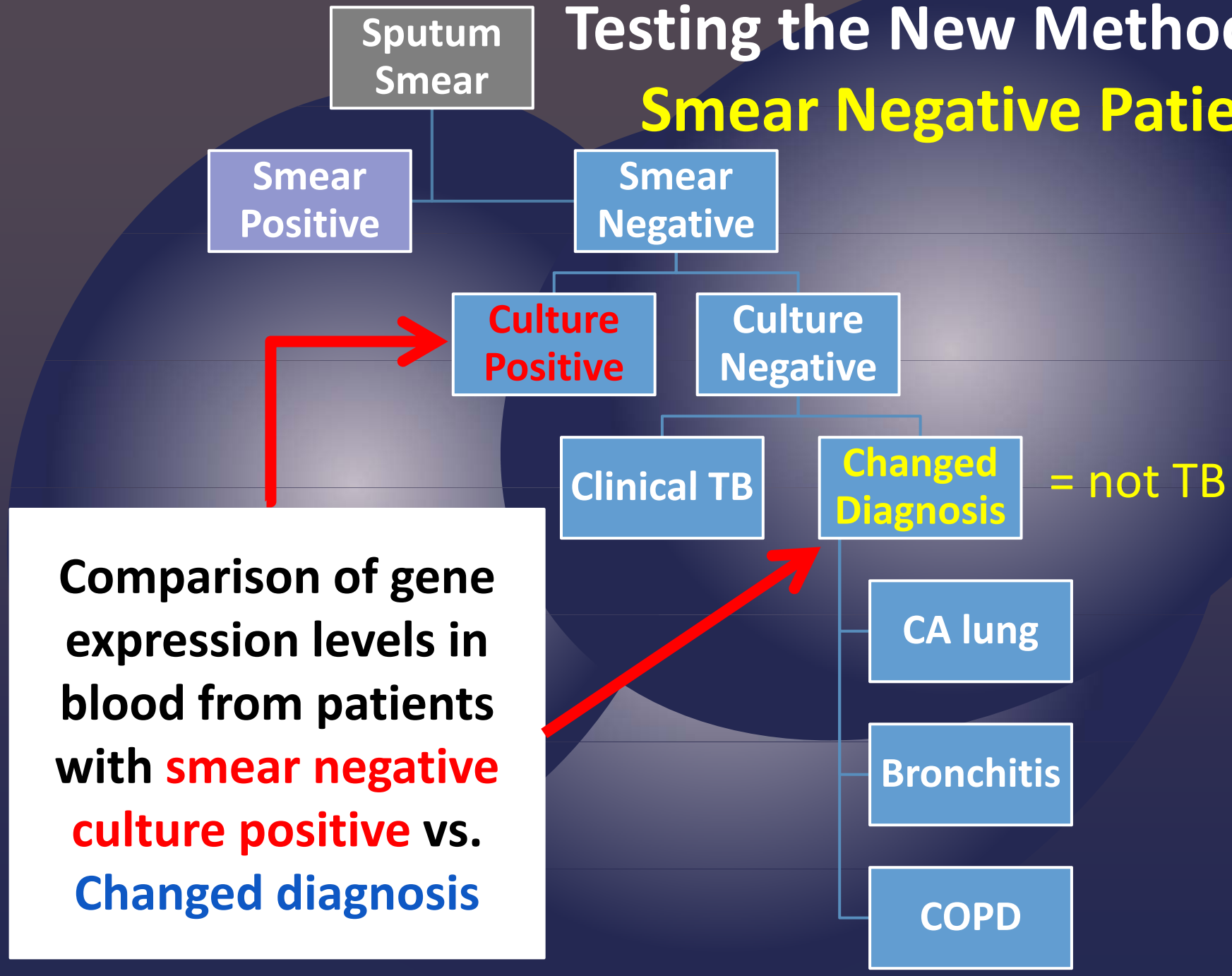
Sensitivity and Specificity of "TB Sick Score" as Compared to Culture Confirmation

Diagnosis Results	TB	Healthy Control	Cured TB	Total
TB (TB sick score ≥ 1.0)	33	0	0	33
Not TB (TB sick score < 1.0)	7	38	18	63
Total	40	38	18	96

Sensitivity 82.5%
Specificity 100%
Positive Predictive Value (PPV) 100%
Negative Predictive Value (NPV) 88.89%
(TB incidence in Thailand = 182/100,000)

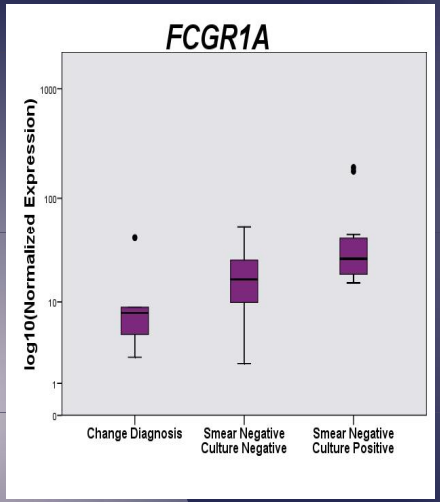
Satproedprai N. et al. 2015 genes and immunity

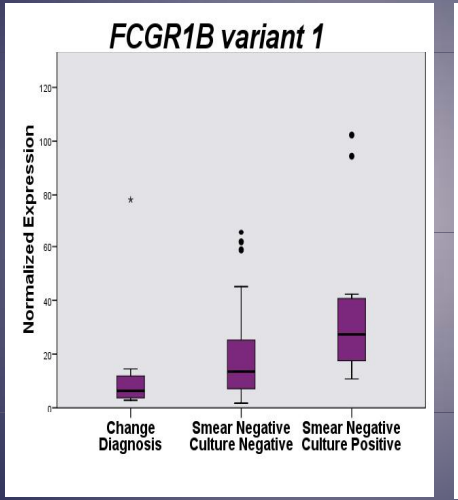
Testing the New Method in Smear Negative Patients

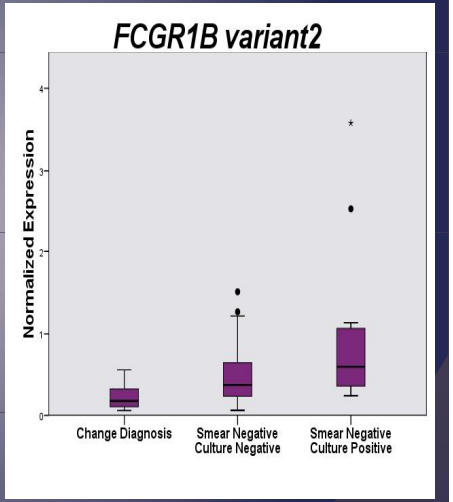


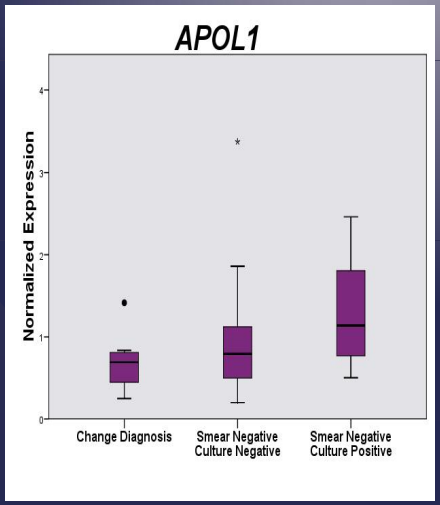
Comparison of Gene Expression Level in Smear Negative TB Cases

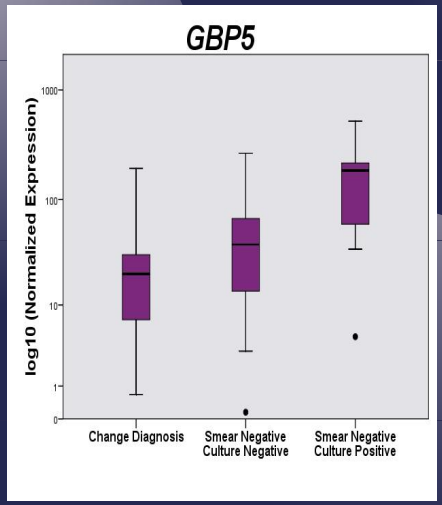
7 out of 9 selected genes showed p value > 0.01 when compare gene expression levels between smear negative culture positive, smear negative culture negative and change diagnosis

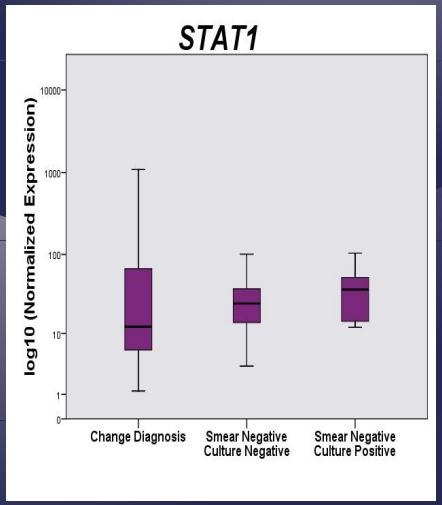


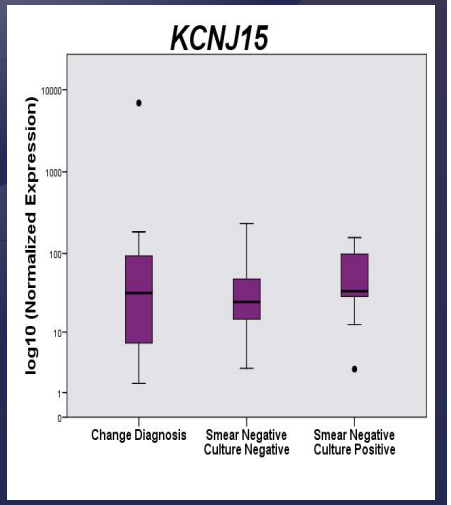












unpublished data

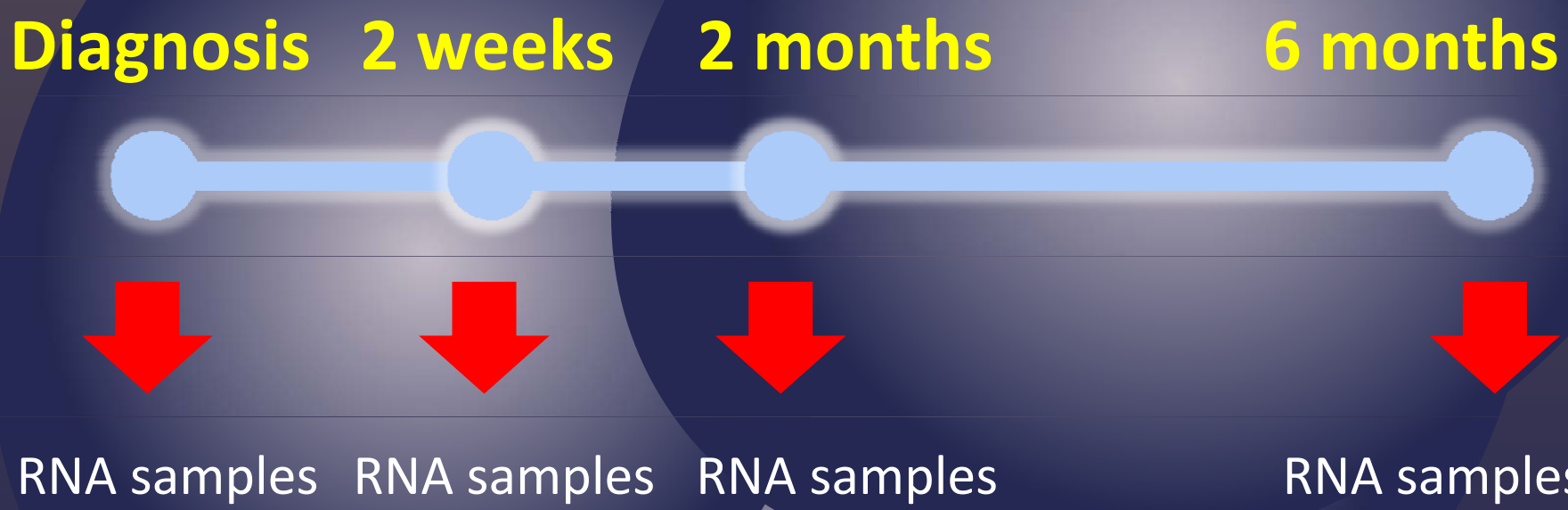
Sensitivity and Specificity of the New Method in Sputum Smear Negative Patients

AUC = 0.951
Sensitivity = 82.6%
Specificity = 100%

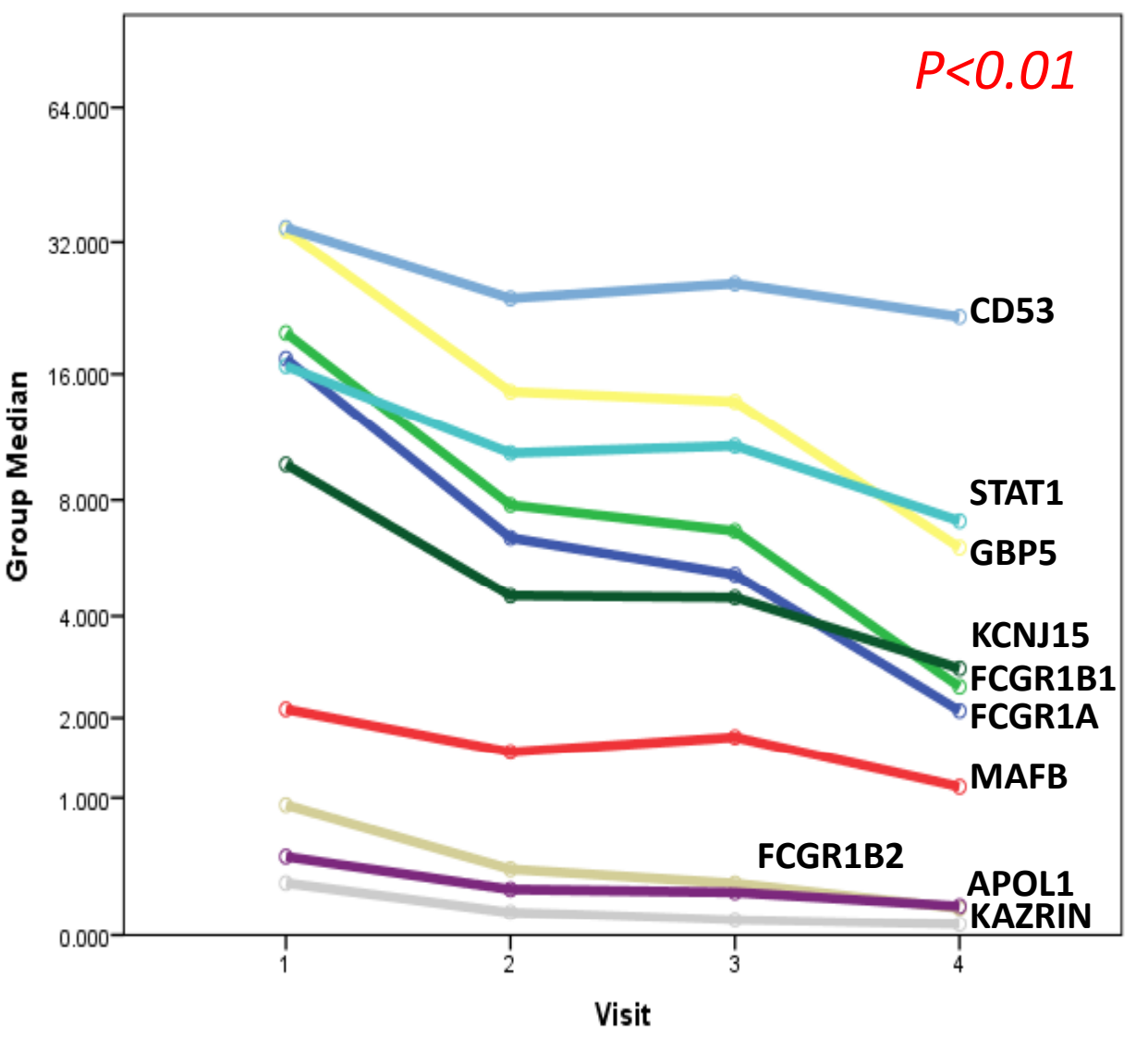
Unpublished data

Gene expression biomarkers and TB treatment monitoring

Standard TB Treatment Course = 6 months



Gene expression levels changes during the course of TB treatment



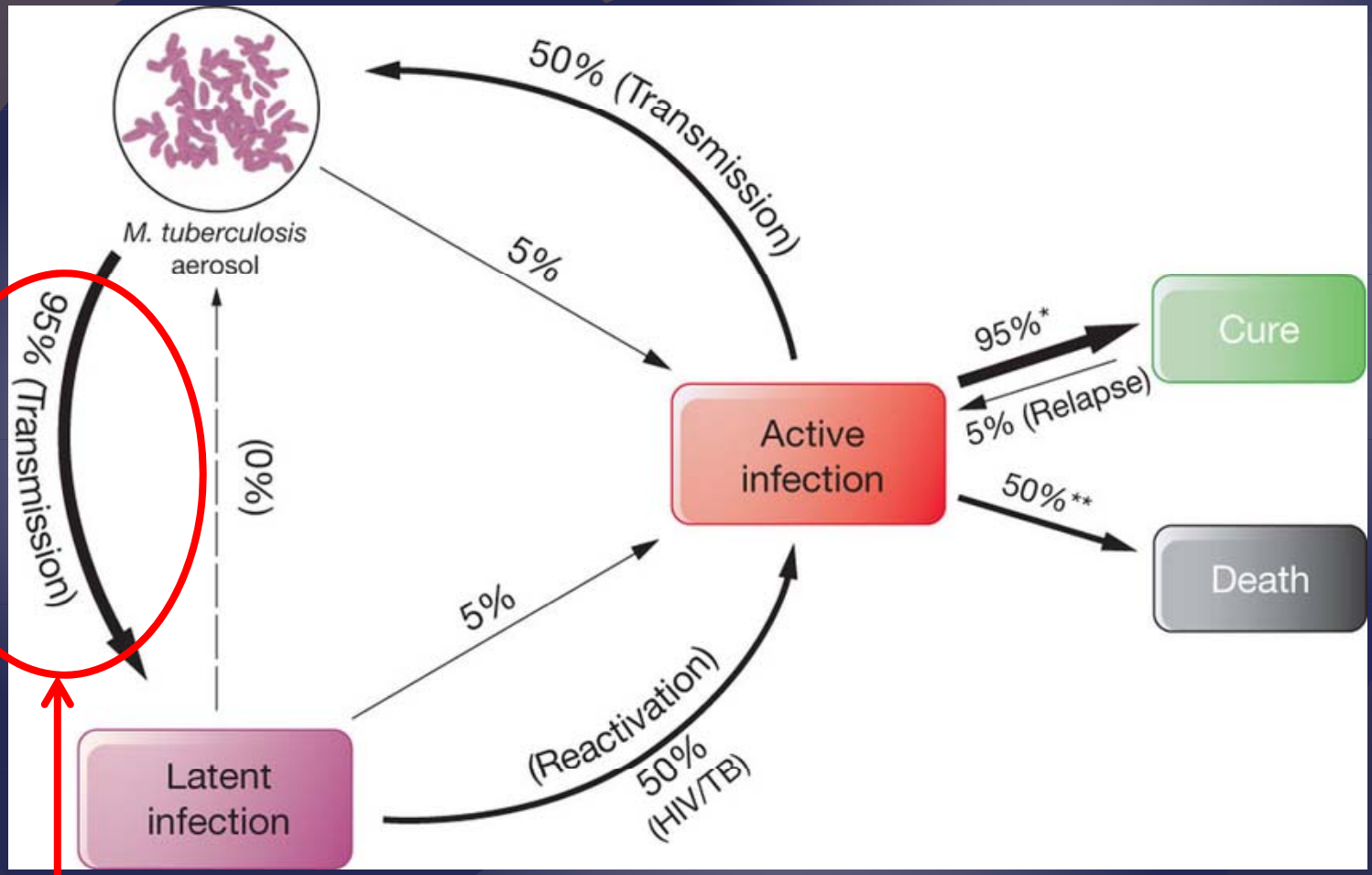
- 18 smear positive TB patients with culture confirmation
- Follow up for 4 visits during TB treatment

Visit 1: at Diagnosis
Visit 2: 2 wk. after treatment
Visit 3: 2 mo. after treatment
Visit 4: Treatment complete

Unpublished data

Gene Expression Biomarkers as a Tool for Stratification of Latent TB

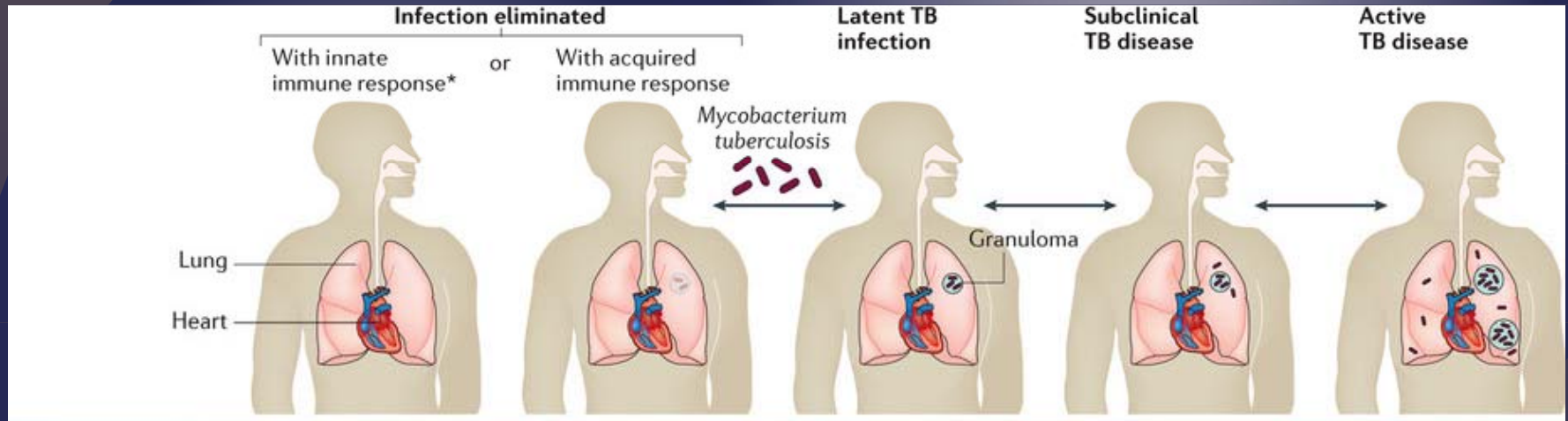
Latent TB – The Key to End TB?



Treatment

Koul A. *et al.* Nature 469(7331):483-90 · January 2011

Can Gene Expression Biomarkers differentiate immune spectrum of LTBI?

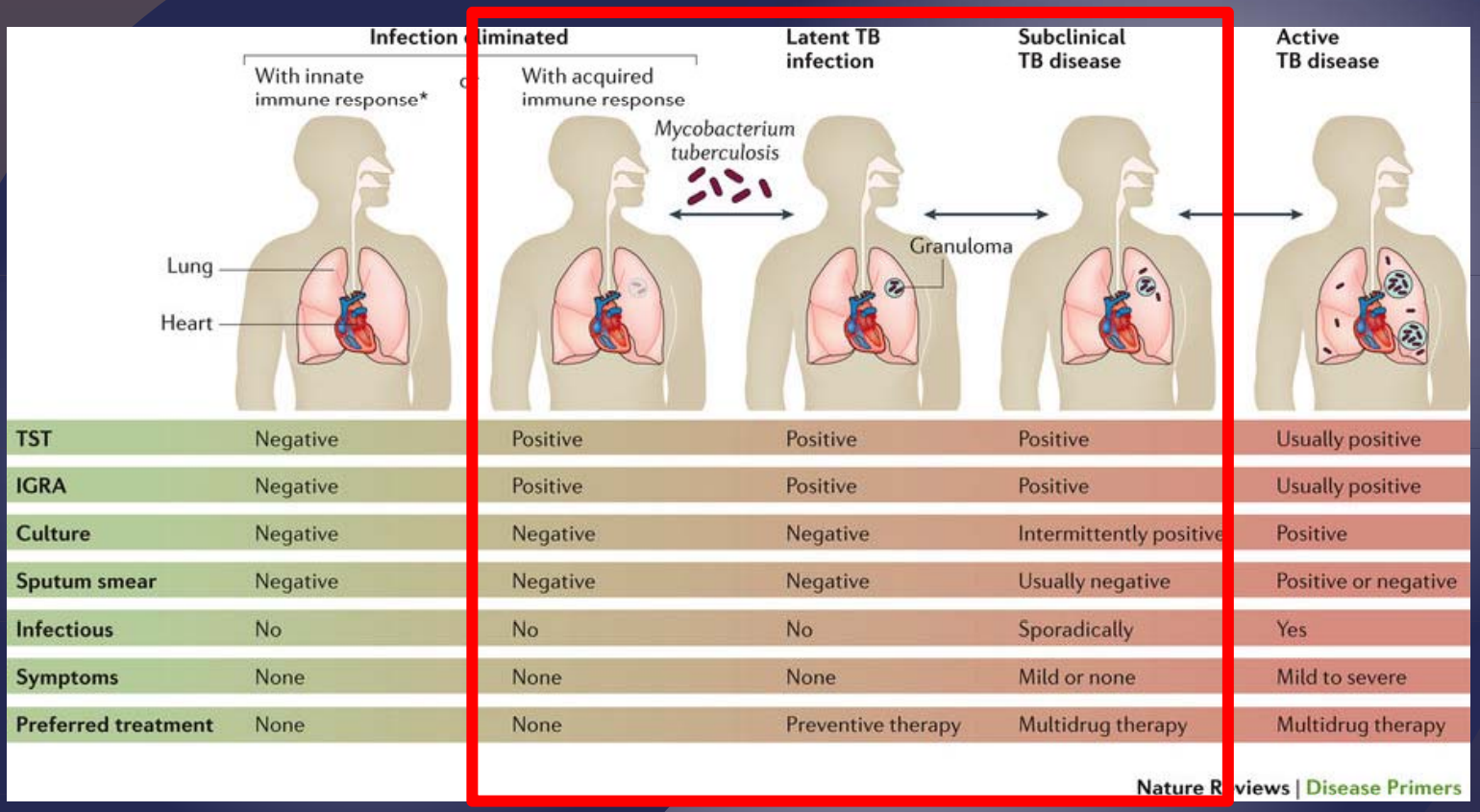


TST	Negative	Positive	Positive	Positive	Usually positive
IGRA	Negative	Positive	Positive	Positive	Usually positive
Culture	Negative	Negative	Negative	Intermittently positive	Positive
Sputum smear	Negative	Negative	Negative	Usually negative	Positive or negative
Infectious	No	No	No	Sporadically	Yes
Symptoms	None	None	None	Mild or none	Mild to severe
Preferred treatment	None	None	Preventive therapy	Multidrug therapy	Multidrug therapy

Nature Reviews | Disease Primers

Pai, M. et al. 2016 Nat. Rev. Dis. Primer

Can Gene Expression Biomarkers differentiate immune spectrum of LTBI?



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Nature Reviews | Disease Primers

IGRA -ve
Chest X-ray Normal

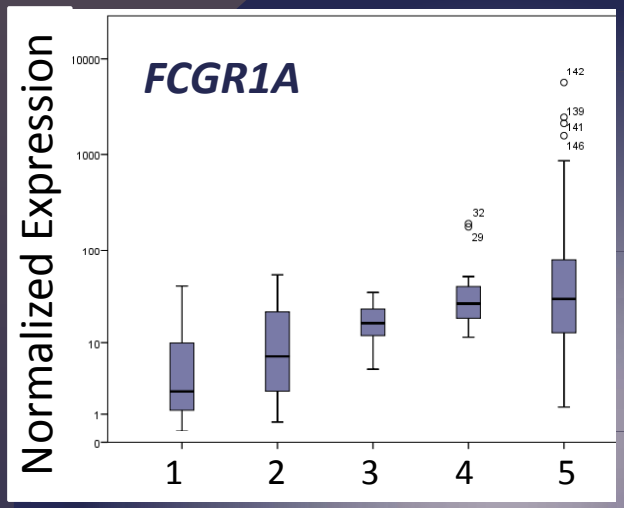
IGRA +ve
Chest X-ray Normal
Sputum -ve
Culture -ve

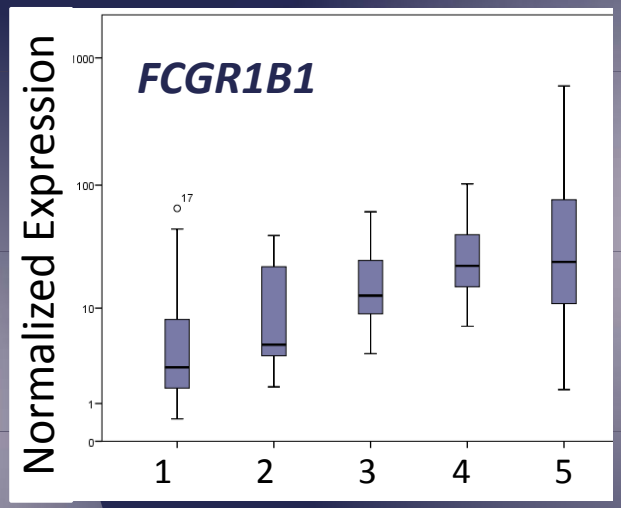
IGRA +ve
Chest X-ray ±
Sputum -ve
Culture -ve

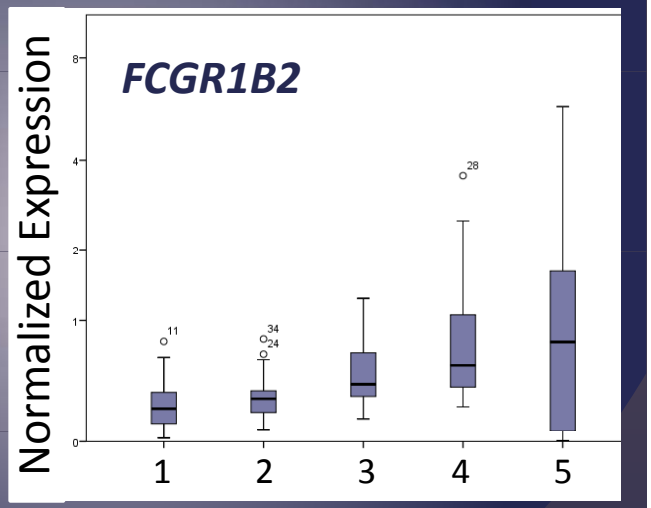
IGRA +ve
Chest X-ray Abnormal
Sputum -ve
Culture +ve

IGRA +ve
Chest X-ray Abnormal
Sputum +ve
Culture +ve

Expression levels of *FCGR1* changes according to the Immune Spectrum of TB infection



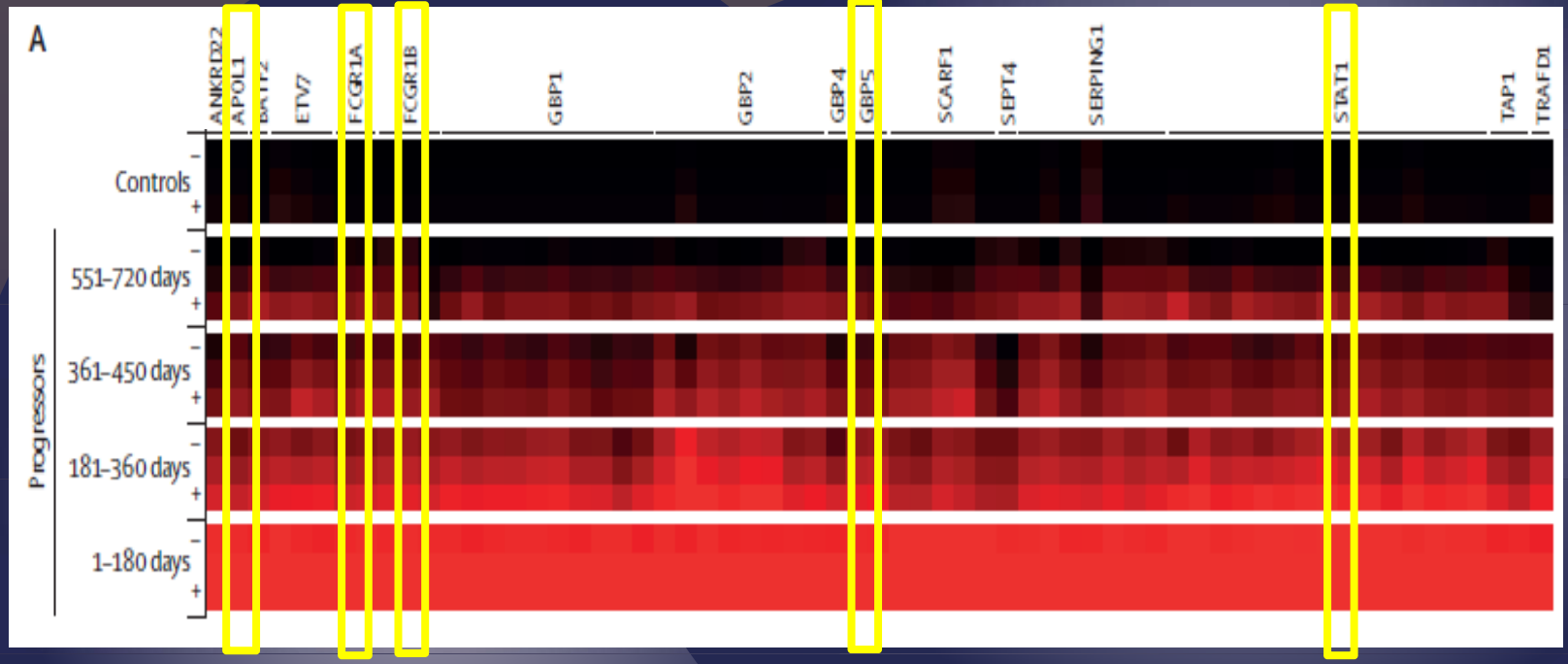




1	2	3	4	5
IGRA -ve Chest X-ray Normal	IGRA +ve Chest X-ray Normal Sputum -ve Culture -ve	IGRA +ve Chest X-ray ± Sputum -ve Culture -ve	IGRA +ve Chest X-ray Abnormal Sputum -ve Culture +ve	IGRA +ve Chest X-ray Abnormal Sputum +ve Culture +ve

Unpublished data

Gene Expression Biomarkers in LTBI



	ROC AUC (95% CI)	Sensitivity (95% CI)	Threshold
By 6 month period			
1–180	0·79 (0·76–0·82)	71·2% (66·6–75·2)	61%
181–360	0·771 (0·75–0·79)	62·9% (59·0–66·4)	61%
361–540	0·726 (0·70–0·76)	47·7% (42·9–52·5)	61%
541–720	0·540 (0·49–0·59)	29·1% (23·1–35·9)	61%
>720	0·496 (0·43–0·56)	5·4% (2·4–13·0)	61%
By 12 month period			
1–360	0·779 (0·76–0·80)	66·1% (63·2–68·9)	61%
360–720	0·647 (0·62–0·673)	37·5% (33·9–41·2)	61%
Total time period	0·743 (0·73–0·76)	58·4% (56·1–60·7)	61%

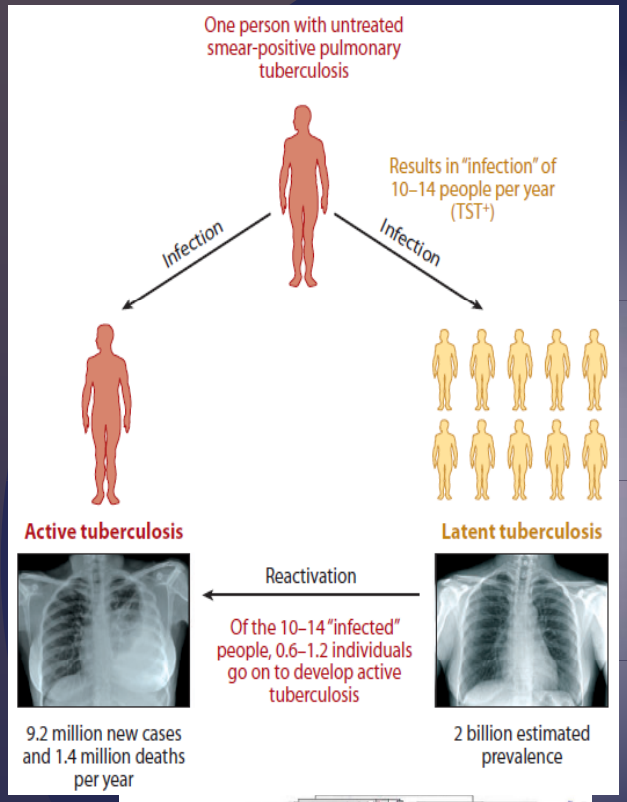
Sensitivity values are reported at a specificity of 80·0% (95% CI 78·6–81·4). ROC AUC=area under receiver operating characteristic curve. ACS=adolescent cohort study.

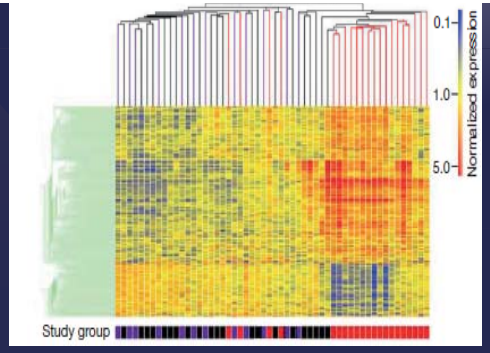
Table 1: Cross-validation performance of the tuberculosis risk signature in the ACS training set by days before tuberculosis diagnosis

- 16 genes transcript can predict Latent TB progression up to 6 months (180 days) in advances with 71.2% sensitivity with overall sensitivity of 58.4% for the total time period of 2 years (720 days)

Zak D.E. et al. 2016 Lancet

Discovery of New Biomarkers for TB Diagnosis





- **Berry MP *et al.* 2010** – identify blood gene expression biomarkers (signatures) in blood that can differentiate Active TB patients from Latent TB and normal control
- **Bloom CL et al. 2012** - report the possibility of using gene expression signatures as a tool to monitor treatment response
- **Kaforou M *et al.* 2013** – Blood gene expression signatures can differentiate Active TB in a HIV-TB co-infection cohort
- **Anderson S. *et al.* 2014** – Confirmed that blood gene expression signatures can also be used in pediatrics TB
- **Satproedprai *et al.* 2015** – Reported the use of 7 genes expression level to accurately differentiate Active TB from Normal and Cured TB
- **Zak DE *et al.* 2016** – Identify 16 genes panel that can predict TB reactivation 6 months in advance
- **Suliman *et al.* 2018** – Using of 4-genes panel to predict up to 2 years of TB onset in Latent TB across 4 African countries

Summary

- ✓ Developed a new diagnostic method for TB using RNA biomarkers

Detect in blood / High Sens. Spec. / Short turnaround time

- ✓ Can be used to assist Active TB Diagnosis in suspected case with smear negative or culture negative
- ✓ Plausible with HIV-TB and Childhood TB
- ✓ May be used to assist diagnosis of active TB in contact investigation and risk stratification for TB or preventive TB Treatment

Acknowledgement

University of Tokyo,
- Prof. Katsushi Tokunaga,
- Dr. Licht Toyo-oka
- Dr. Yosuke Omae

RIKEN
- Dr. Taisei Mushiroda

Japan Anti-tuberculosis Association (JATA)
- Dr. Takashi Yoshiyama
- Dr. Naoto Keicho
- Dr. Hideki Yanai

Mahidol University:
- Prof. Prasit Palittapongarnpim
- Assoc.Prof. Angkana Chaiprasert
- Dr. Pravech Ajawatanawong
- Dr. Pornpen Tantivitayakul

NSTDA (National Science and Technology Development Agency):
- Dr. Therdsak Prammananan
- Dr. Wasna Viratyosin
- Dr. Nat Smittipat

Department of Medial Sciences, MoPH
- Dr. Surakameth Mahasirimongkol
- Dr. Nuanjun Wichukchinda
- Dr. Archawin Rojanawiwat
- Dr. Panadda Dhepakson
- Dr. Nusara Satproedprai

Department of Disease Control, MoPH
- Dr. Petchawan Puengrassami
- Dr. Phalin Kramolwat
- Ms. Saijai Smithtikarn
- Dr. Niramon Pimnamyen
- Ms. Phikul Tipkrua

Central Chest Institute of Thailand, Department of Medical Services, MoPH
- Dr. Charoen Chuchottaworn
- Dr. Narumon Luekittinun

Chiangrai Prachanukroh Hospital
- Dr. Worarat Imasanguan
- Dr. Supalert Nedsuwan

All the collaborators and patients in this project

These researches are supported by SATREPs (AMED/JST)