Blood transcriptomics for TB diagnosis and monitoring treatment-response

Dr Jackie Cliff

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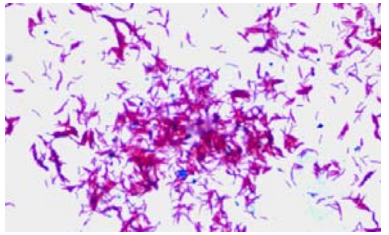
How can blood transcriptomics help?

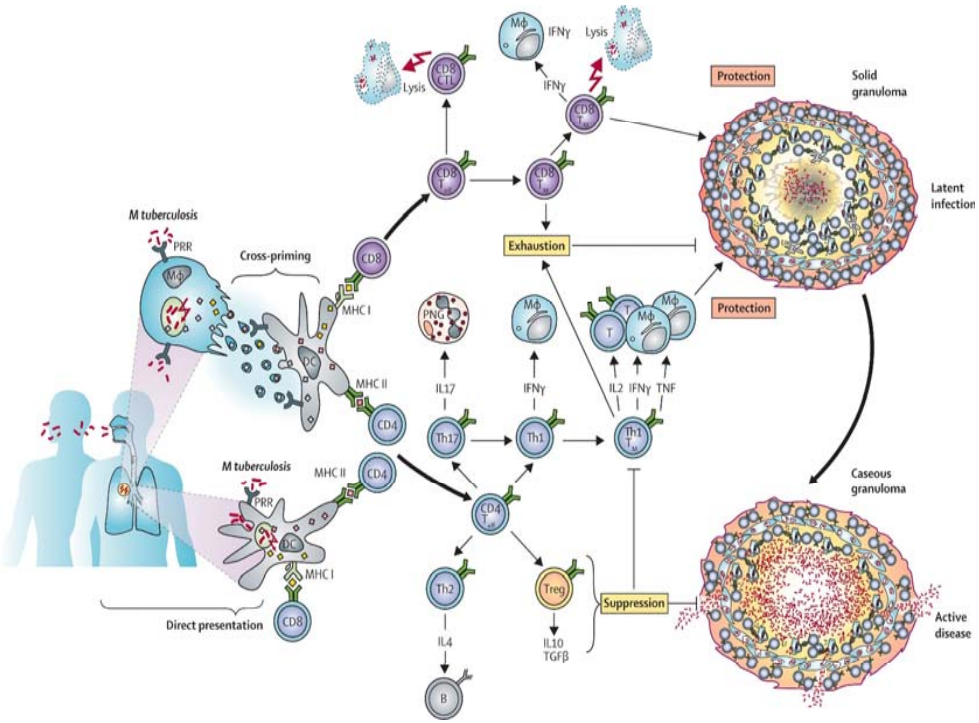


- Improved diagnostics for tuberculosis – more rapid testing

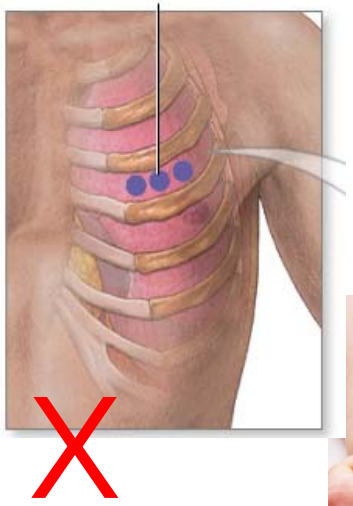
- Measurement of drug treatment efficacy
  - Clinical trials of new drugs or regimens
    - Shortened treatment
    - Anti-microbial resistance
    - Latent TB treatment
  - Stratified medicine

- Stratification in vaccine trials

In order to develop biomarkers for clinical use, we need to use an accessible sample



Kaufmann *et al*, *The Lancet* 2010 375, 2110-2119



First question: can we detect different stages of infection using blood transcriptomics?

UNIVERSITEIT STELLENBOSCH UNIVERSITY

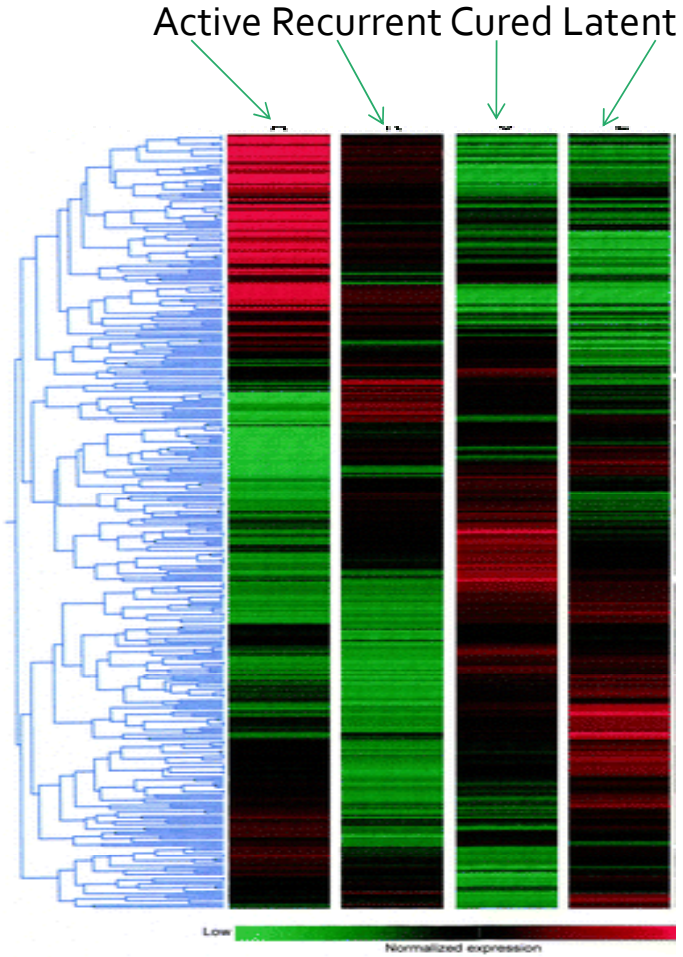
MAJOR ARTICLE

Gene-Expression Patterns in Whole Blood Identify Subjects at Risk for Recurrent Tuberculosis

Rohit Mistry,[1,3] Jacqueline M. Cliff,[2] Christopher L. Clayton,[1] Nulda Beyers,[4] Yasmin S. Mohamed,[1] Paul A. Wilson,[1] Hazel M. Dockrell,[2] Don M. Wallace,[1] Paul D. van Helden,[3,4] Ken Duncan,[1,a] and Pauline T. Lukey[1]

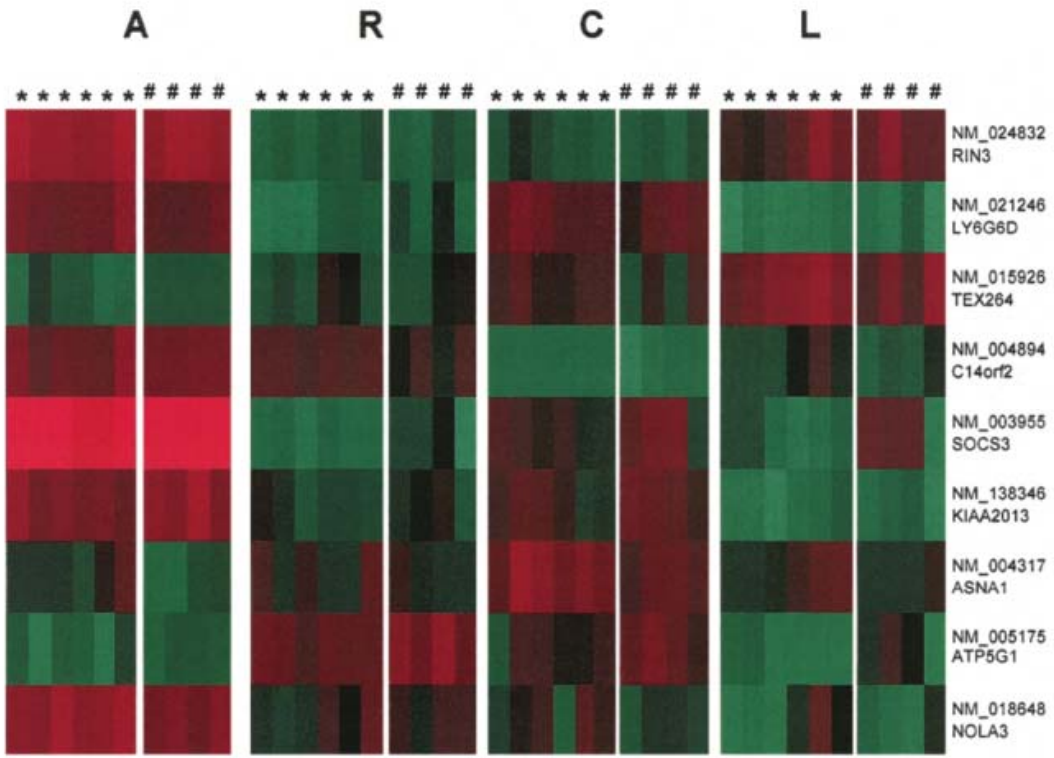
[1]GlaxoSmithKline, Stevenage, and [2]Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom; [3]Department of Medical Biochemistry and [4]Desmond Tutu TB Centre, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa

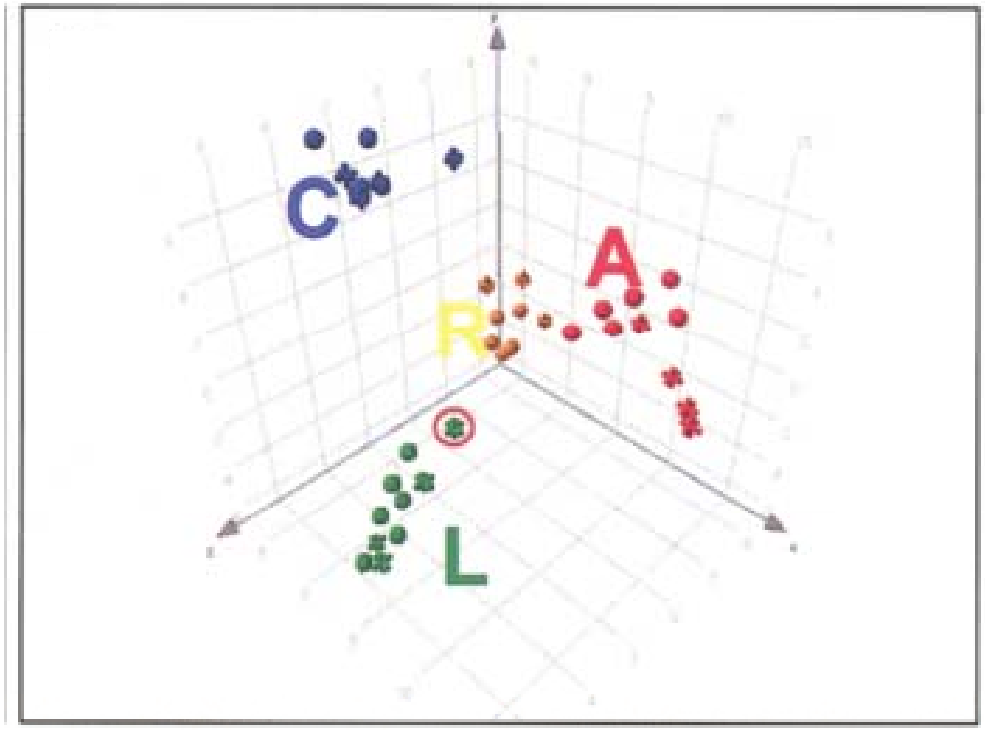
J Infect Diseases 2007



n = 10 per group

An algorithm based on expression of only 9 genes could discriminate the groups





Similar results have been found by other researchers

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PLOS | ONE

Genome-Wide Expression Profiling Identifies Type 1

Tom H.
Edhyan
Sangko

1 Infectious
Netherlands
Bandung, In

J Mol Med (2007) 85:613–621
DOI 10.1007/s00109-007-0157-6

ORIGINAL ARTICLE

Candidate bioma
and disease cause

Marc Jacobsen · Dirk Repsilbe
Albert Neher · Knut Feldmann
Andreas Ziegler · Stefan H. E.

Vol 466|19 August 2010|doi:10.1038/nature09247

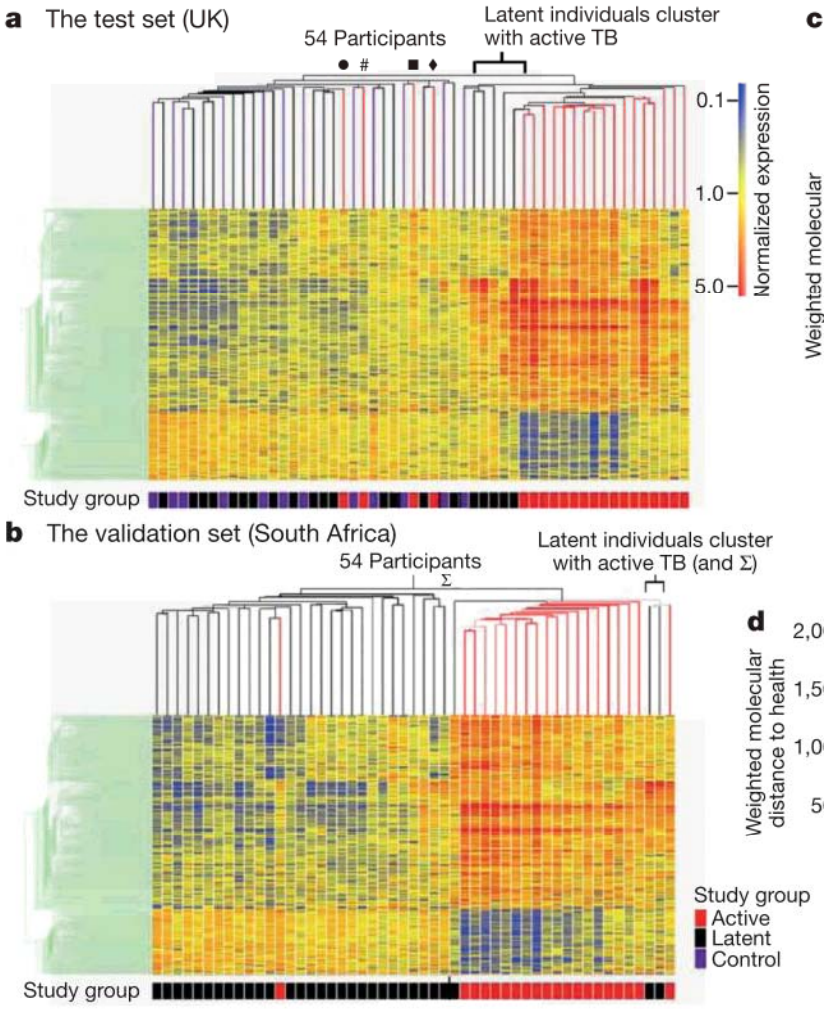
nature

LETTERS

An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis

Matthew P. R. Berry[1], Christine M. Graham[1]*, Finlay W. McNab[1]*, Zhaohui Xu[6], Susannah A. A. Bloch[3], Tolu Oni[4,5], Katalin A. Wilkinson[2,4], Romain Banchereau[9], Jason Skinner[6], Robert J. Wilkinson[2,4,5], Charles Quinn[6], Derek Blankenship[7], Ranju Dhawan[8], John J. Cush[6], Asuncion Mejias[10], Octavio Ramilo[10], Onn M. Kon[3], Virginia Pascual[6], Jacques Banchereau[6], Damien Chaussabel[6] & Anne O'Garra[1]

The Interferon-inducible neutrophil driven signature



Identified a 393-gene signature, which discriminates Active TB from health

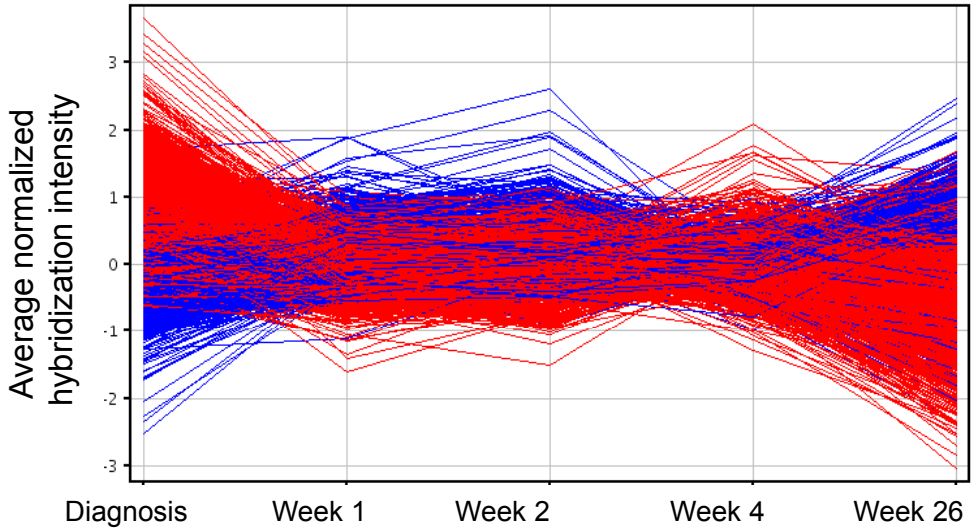
Can assign individuals to groups

Berry, O'Garra et al, Nature 2010

In Active TB, blood transcriptomes revert to health during treatment

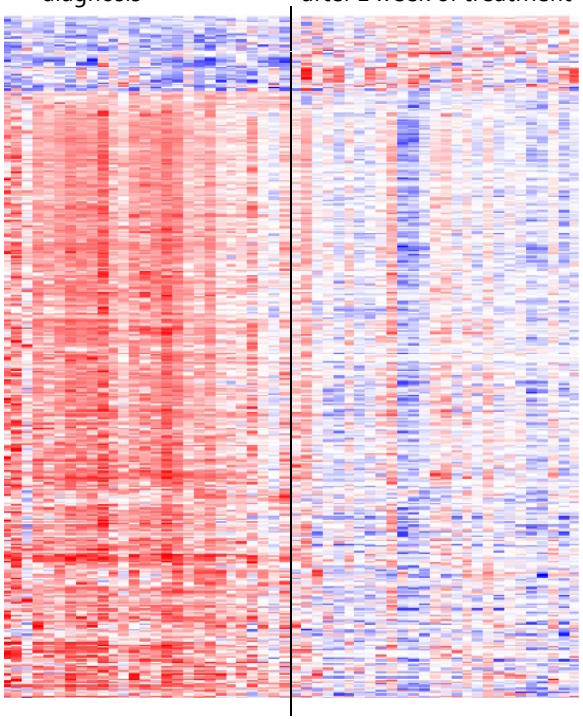
27 active TB cases recruited at Stellenbosch University, Cape Town, successfully cured by conventional treatment

Average changes in gene expression over 6 months



Cliff et al, 2013, *J Infect Dis*

Individual changes over 1st week



Gene expression changes early and late reflect different phases of treatment and disease resolution

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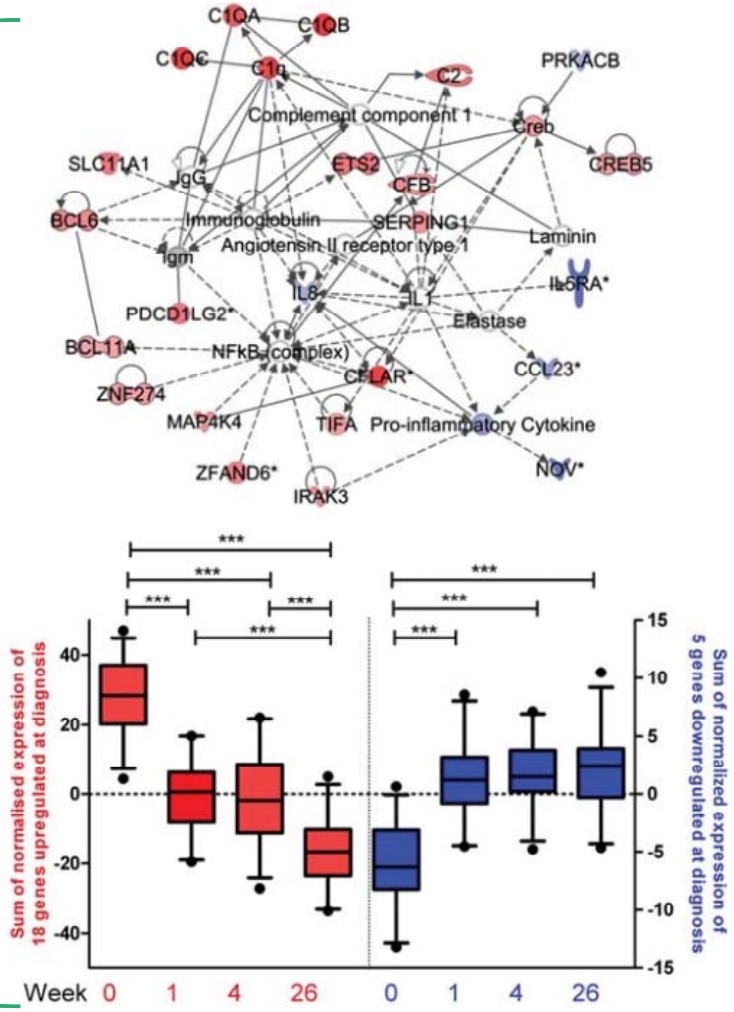
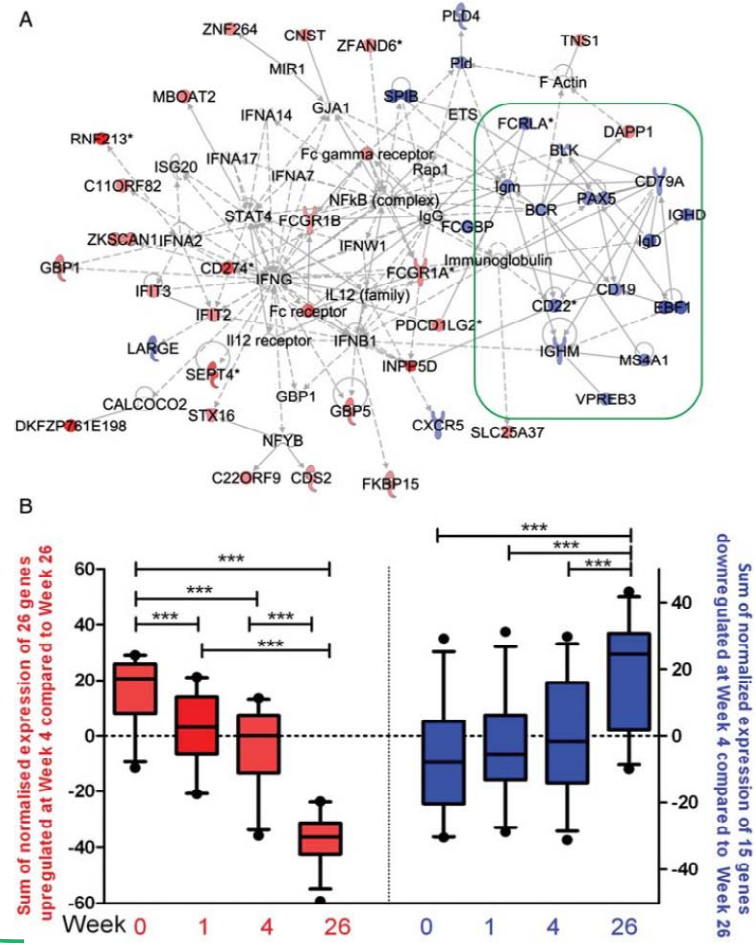
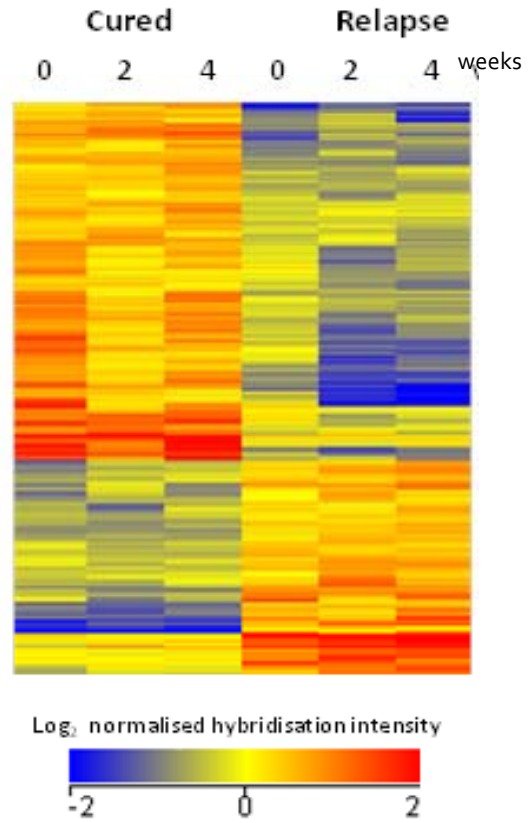
Early changers



Late changers



Prediction of TB-relapse after apparently successful cure



Cliff et al, 2016, *J Infect Dis*

Patients recruited at diagnosis of first episode of TB, then followed up to see who subsequently relapsed

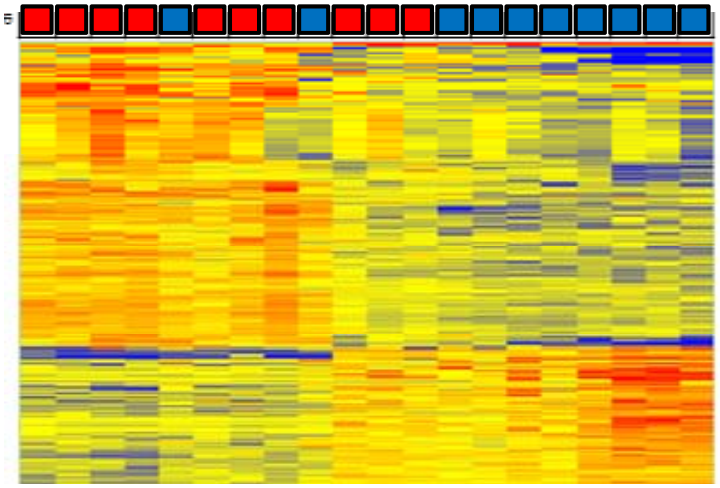
Diluted whole blood, stimulated with *M. tuberculosis* for 6 days

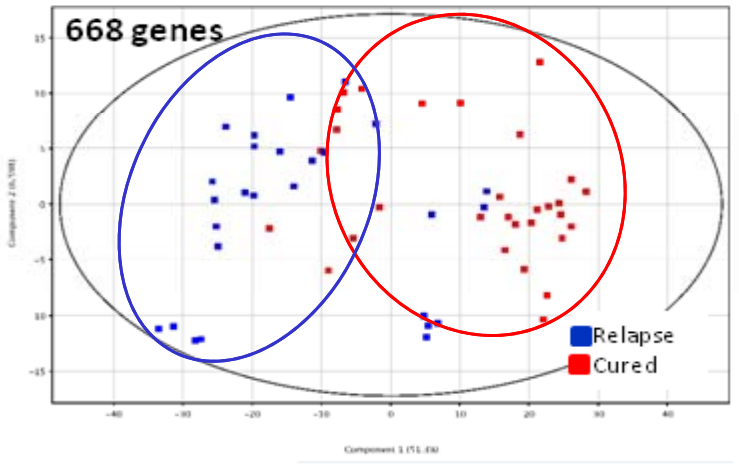
10 Patients who remained Cured for 2 years follow-up

10 Patients who suffered TB-relapse within 2 years

668 genes consistently differentially expressed between relapse and cured patients in ANOVA

A reduced signature is more useful for

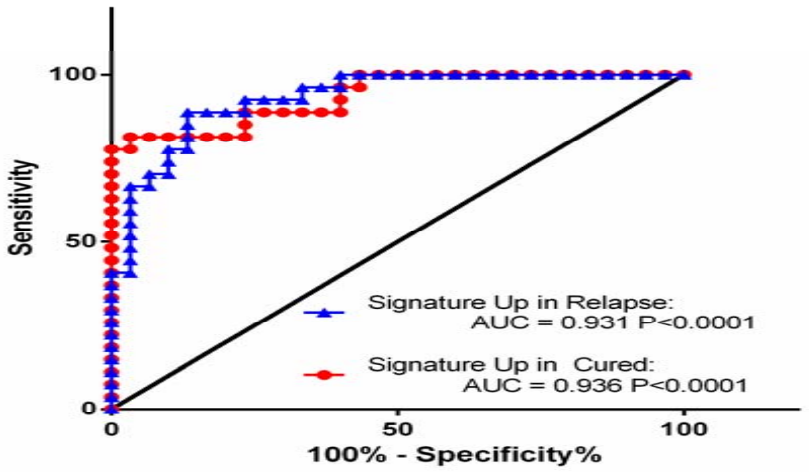




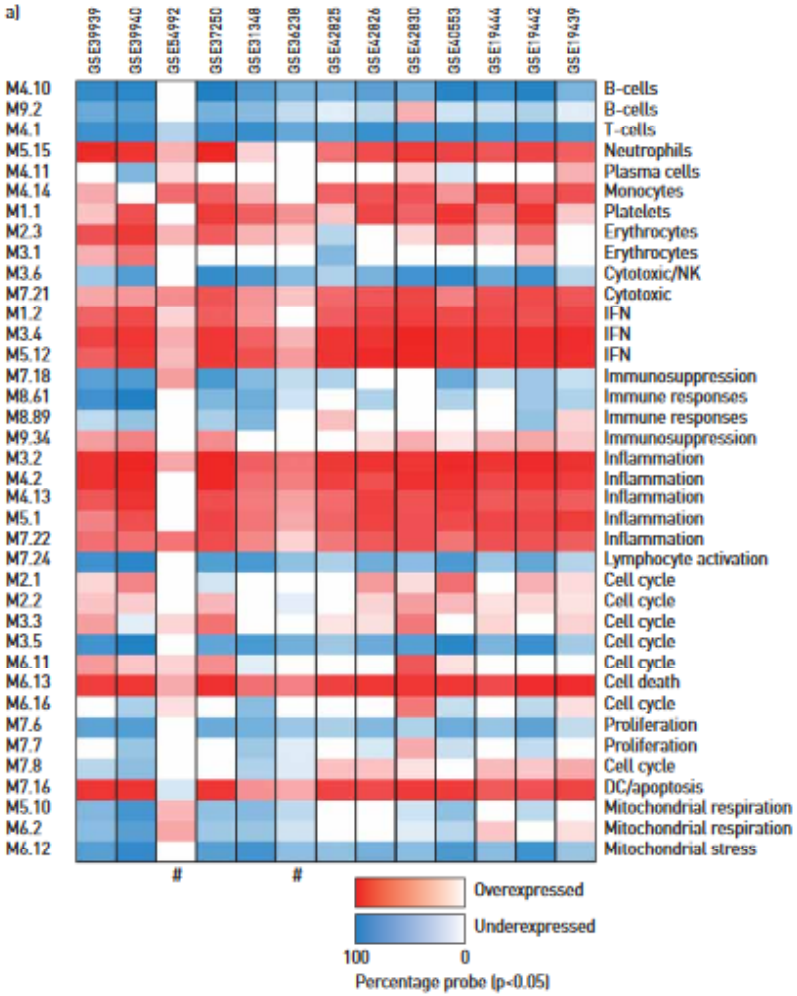
Prediction of relapse can be achieved with 668 genes in most individual patients

An 18 gene signature, based on the most stringently differentially expressed genes in ANOVA, has high predictive sensitivity and specificity

Largely due to excessive cytolytic response in Relapse patients



Studies are reproducible across settings and technology platforms

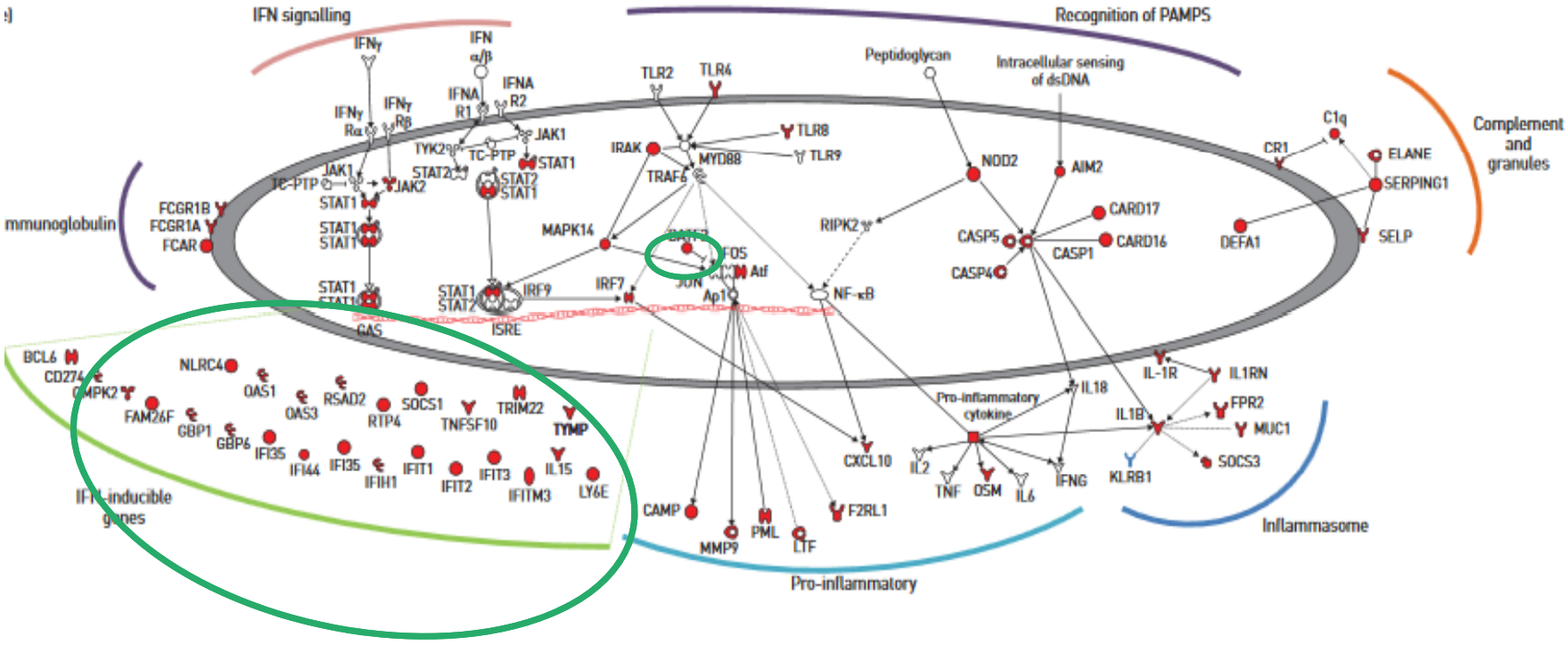


Modular analysis has shown that most datasets contain common signatures

But most studies only include uncomplicated pulmonary TB cases

Blankley, O'Garra et al 2016

Common pathways across tuberculosis patient groups



Blankley, O'Garra et al 2016

Landmark study which incorporated non-straight forward tuberculosis

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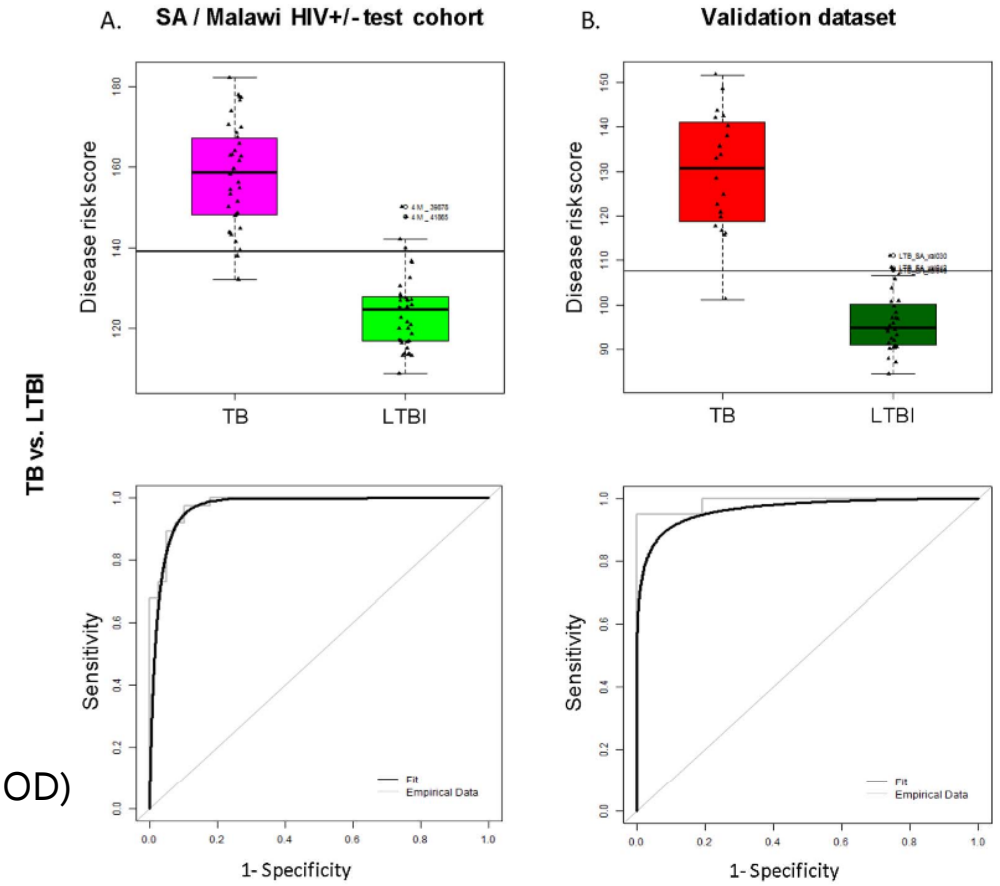
PLOS | MEDICINE

Detection of Tuberculosis in HIV-Infected and -Uninfected African Adults Using Whole Blood RNA Expression Signatures: A Case-Control Study

Myrsini Kaforou[1,2¶], Victoria J. Wright[1¶], Tolu Oni[1,3¶], Neil French[4,5,6¶], Suzanne T. Anderson[7,8], Nonzwakazi Bangani[3], Claire M. Banwell[7,8], Andrew J. Brent[1,9], Amelia C. Crampin[4,6], Hazel M. Dockrell[10], Brian Eley[11], Robert S. Heyderman[8,12], Martin L. Hibberd[13], Florian Kern[7], Paul R. Langford[1], Ling Ling[13], Marc Mendelson[14], Tom H. Ottenhoff[15], Femia Zgambo[4], Robert J. Wilkinson[1,3,16¶], Lachlan J. Coin[2,17¶], Michael Levin[1¶*]

Case-control cohorts in South Africa and Malawi
584 patients with
culture-confirmed TB
or other disease, TB considered (OD)
or LTBI

"Kaforou signatures" – 44-gene (TB vs LTBI) or 27-gene (TB vs OD)



Summary part I

Blood transcriptomes can distinguish TB from healthy controls and from people with other diseases, and give early indication of disease

These signatures can also be used to monitor TB treatment-response, and with stimulation, potentially to predict TB-relapse

People are developing diagnostic tools based on large or small signatures

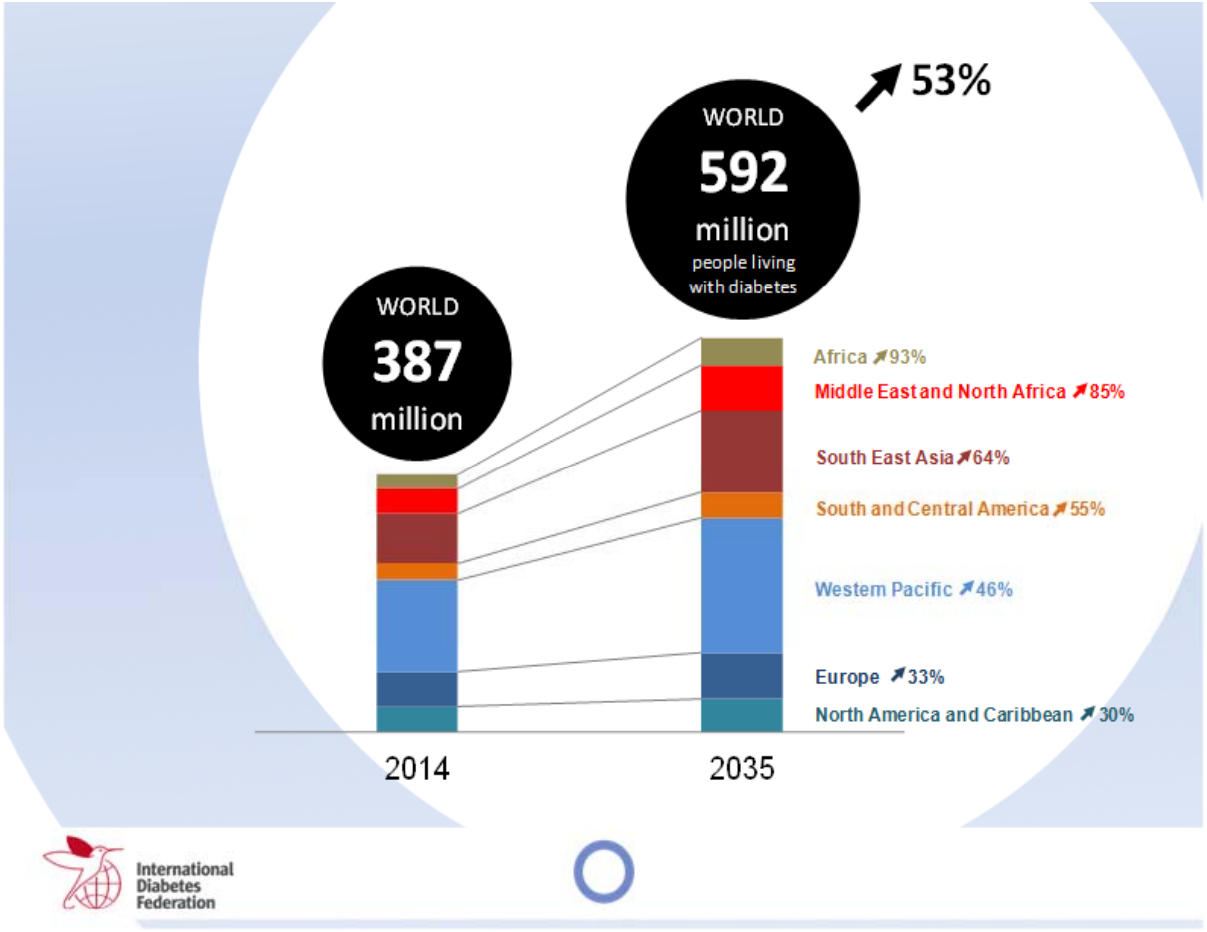
To really be useful, transcriptomic signatures need to be tested and developed in complex conditions

TB-diabetes co-morbidity

People with type 2 diabetes have a 3-fold increased risk of developing active TB once infected

Recent evidence – more likely to become infected

More likely to suffer poor TB treatment outcomes, including death, relapse and treatment failure



Overlap of the TB and T2DM epidemics

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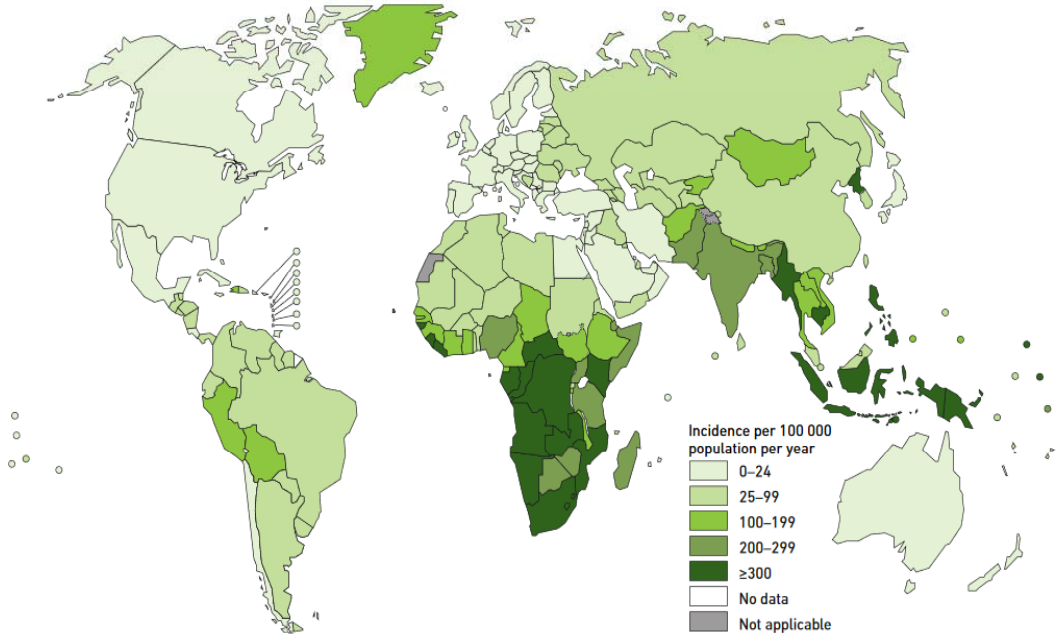
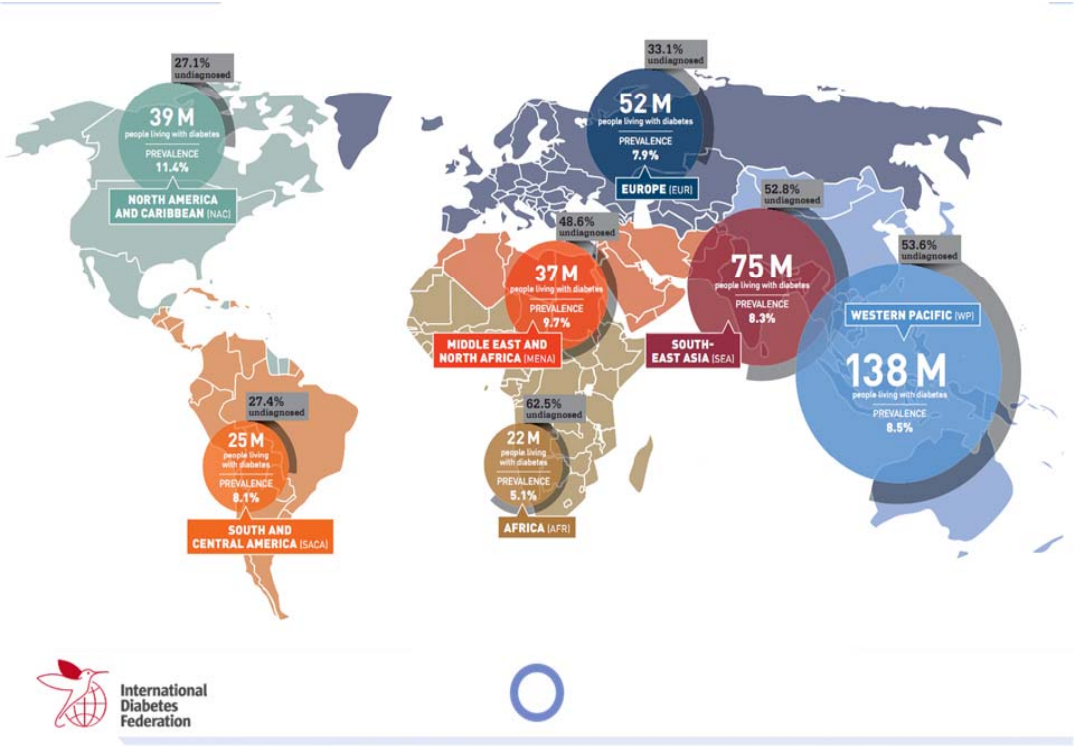
Tuberculosis

estimated incidence



Type 2 Diabetes

estimated prevalence



The TANDEM Study

PI: Hazel Dockrell, LSHTM

University of Medicine and Pharmacy of Craiova, Romania

Lima, Peru

Universidad Peruana Cayetano Heredia

Stellenbosch University, South Africa

Universitas Padjadjaran, Indonesia

RNA-Seq analysis – cross-sectional study

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"complex design"

	TB only	DM only	DM-TB	IH-TB	Healthy controls
South Africa	11	33	15	20	24
Indonesia	14	-	19	5	-
Romania	10	19	15	10	12
Peru	11	-	12	9	-
TOTAL	46	52	61	44	36

All TB patients were microbiologically confirmed pulmonary TB patients

Exclusions: HIV+, other serious co-morbidity, corticosteroid treatment

Samples collected prior to TB treatment commenced

Age, gender, ethnicity not different

Ex vivo blood samples collected into PAXgene tubes

Analysed by RNA-Seq

Clare Eckold
Cisca Wijmenga, Vinod Kumar,
University of Groningen Medical Centre

Intermediate hyperglycaemia

Normal glycaemia:

- HbA1c < 5.7%
- Fasting Plasma Glucose < 100mg/dl

Diabetes:

- HbA1c ≥ 6.5%
- FPG ≥ 126 mg/dl

"Intermediate Hyperglycaemia"

- In between

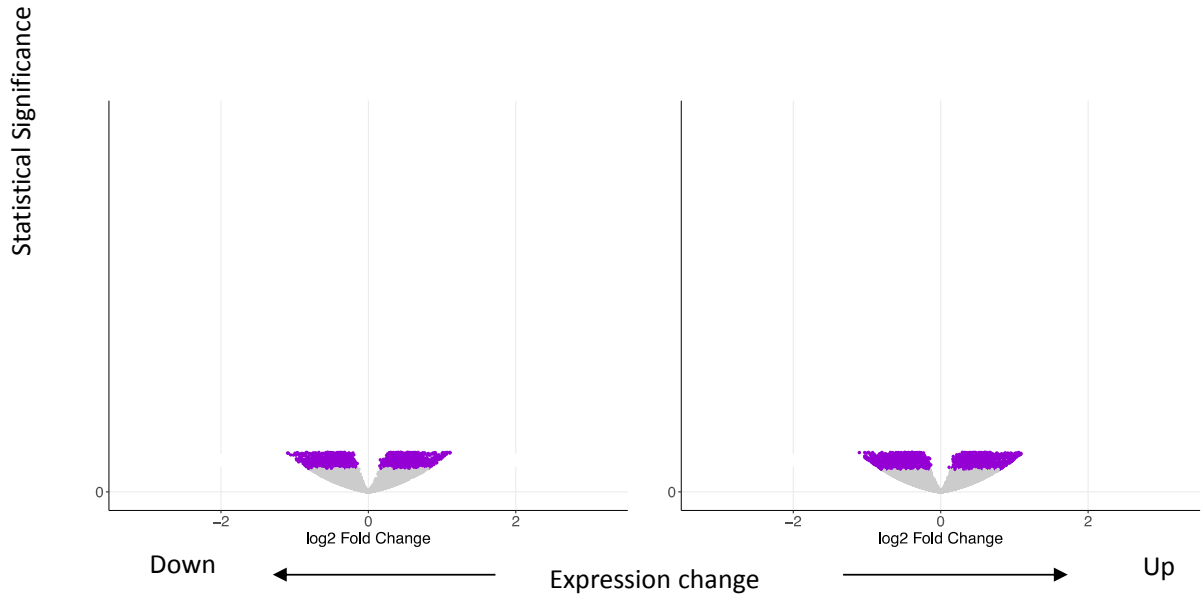
Hypothesis/Expectations

TB is associated with a pro-inflammatory condition, upregulation of myeloid inflammatory genes

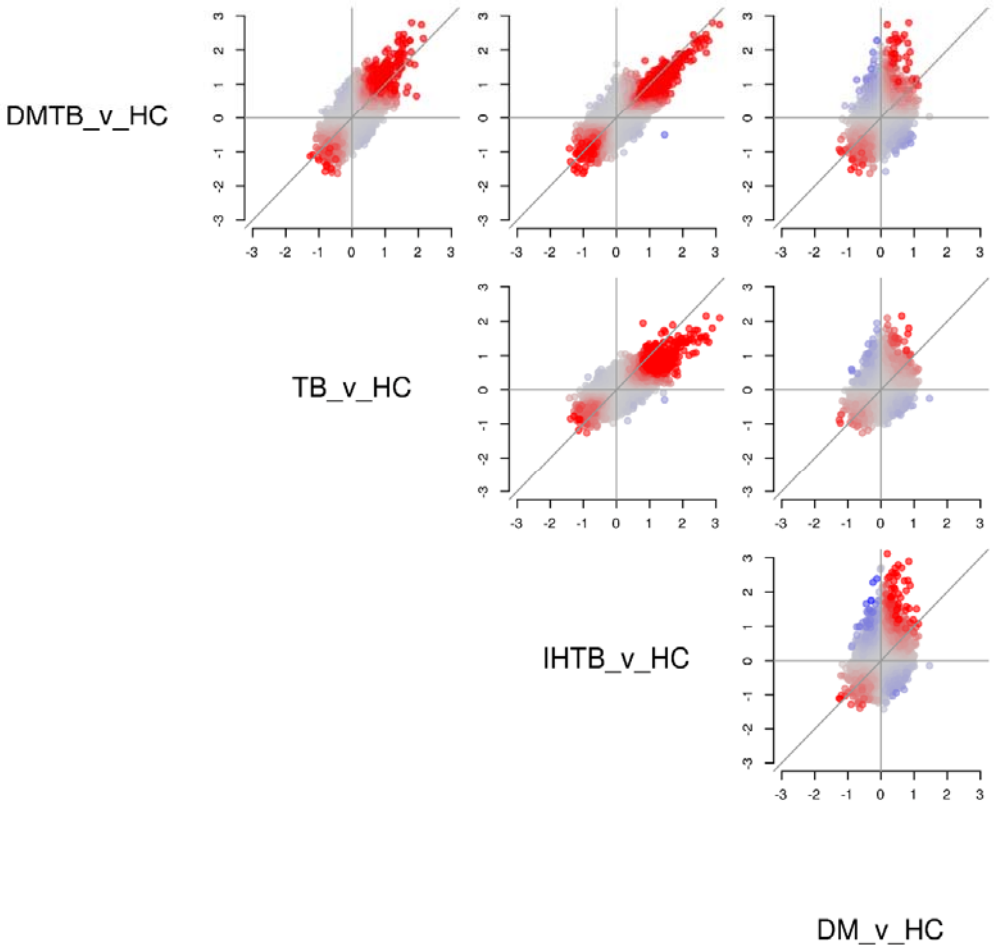
T2DM is also associated with increased inflammation

Hypothesis – people with T2DM – TB would have an exacerbated pro-inflammatory phenotype, which may cause excessive pathology relative to bacterial clearance

South African cohort, relative to Healthy controls



Comparison of comparisons for South Africa



The genes differentially expressed in DM are different to those differentially expressed in TB

The TB signature is dominant and amplified in DM

Principal component analysis of South Africans at baseline

The patients with DM-TB or IH-TB group together, distinctly from patients with only TB

PC1: 34% variance

Modular analysis of expression variation in South Africans

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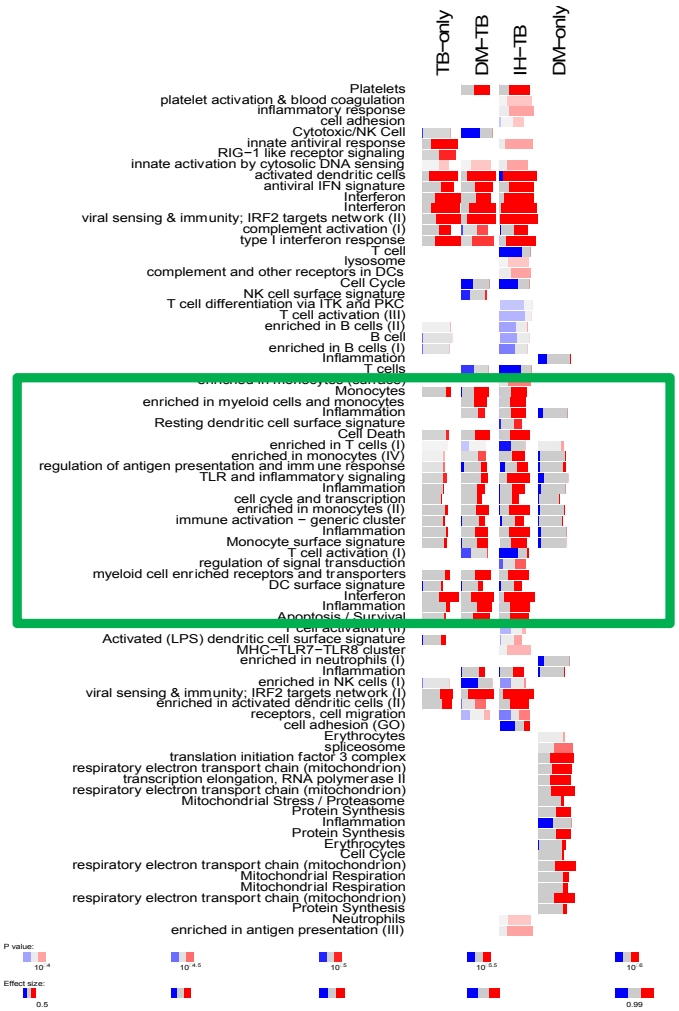
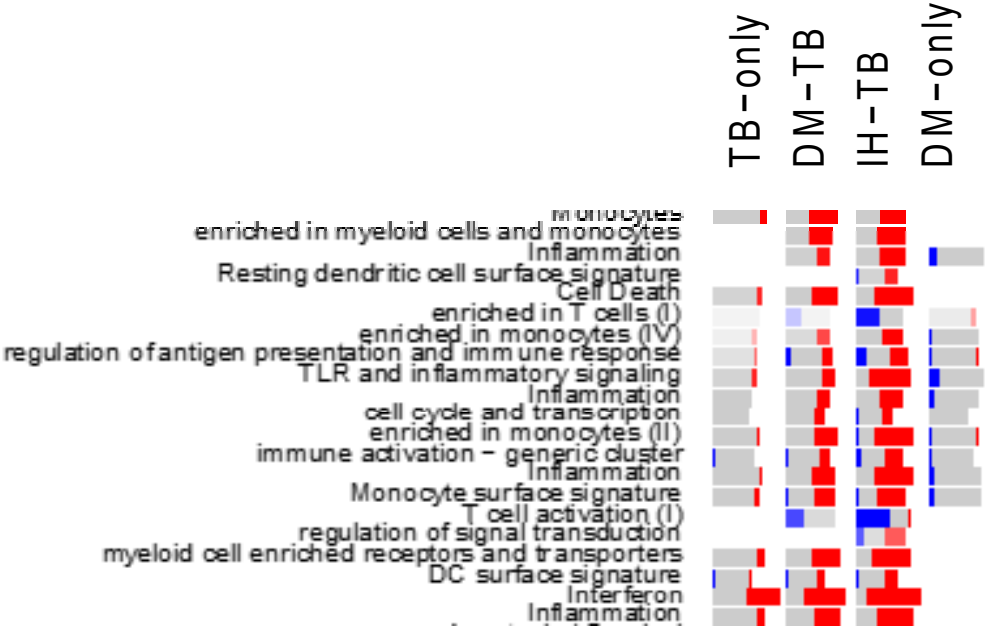
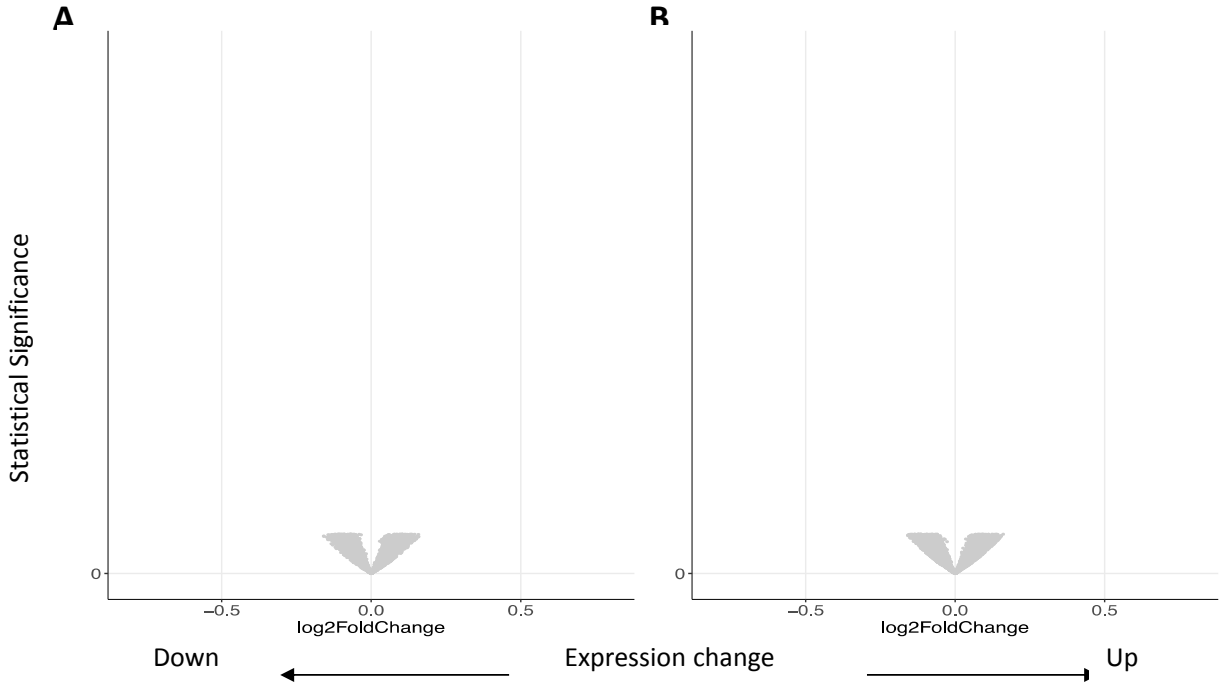




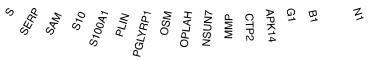
Combined analysis of all field sites



Samples from Romania, Indonesia, Peru and South Africa

All relative to TB-only

Summary of modular analysis in four sites



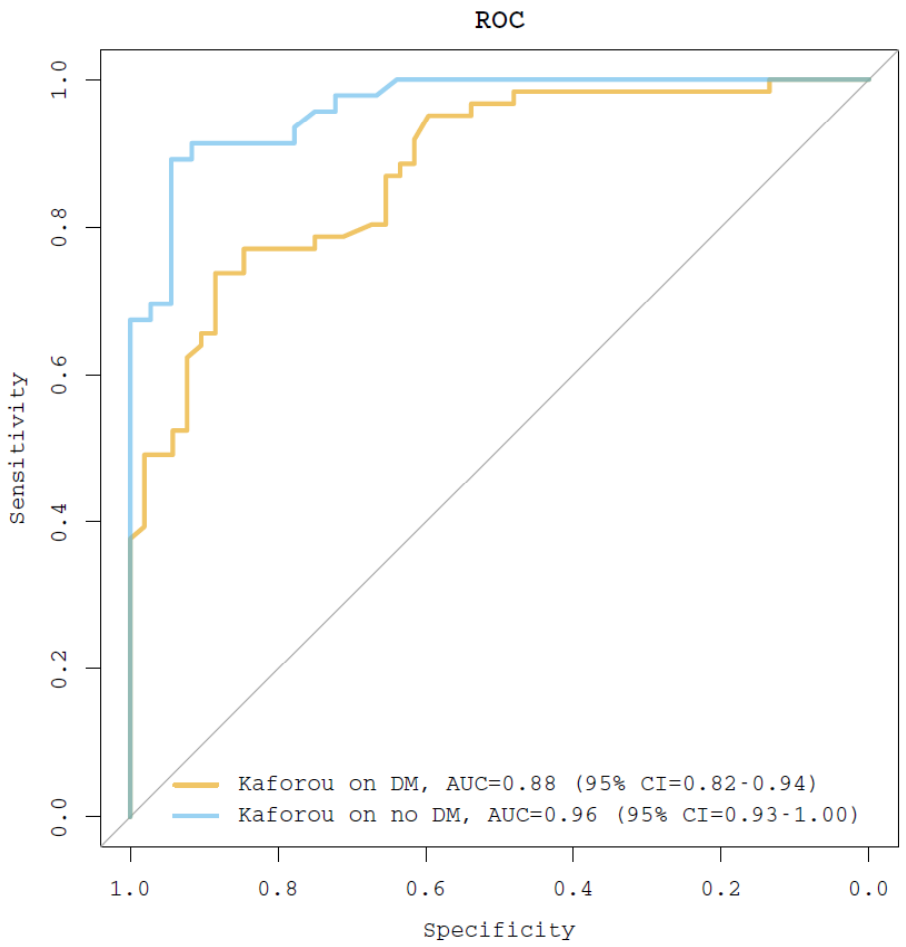
Inside: IHTB; outside DMTB

Across all four populations, there was a decreased interferon signature in diabetes-related TB

The inflammation-related modules were further enhanced

"TB signature" has become uncoupled

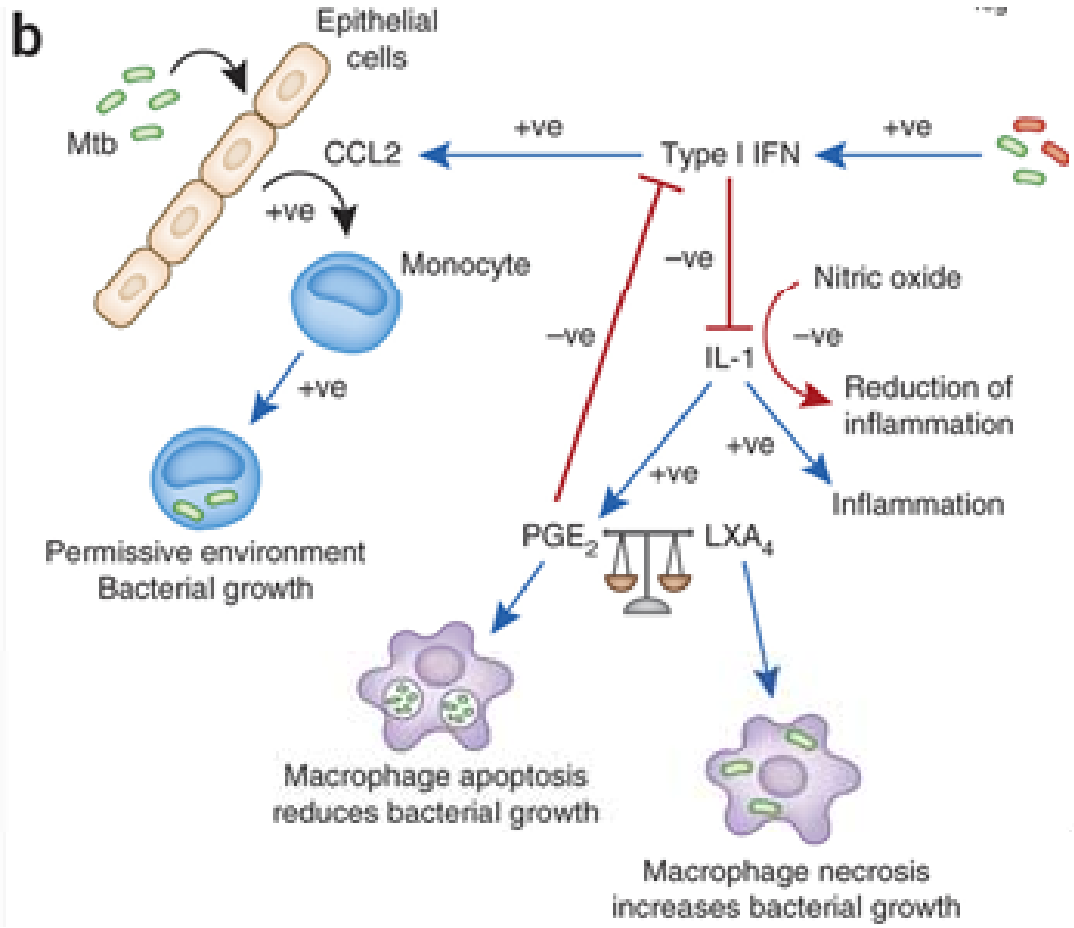
“TB signatures” should be adapted to consider DM



The Kaforou TB signature performed well on the TANDEM samples from TB-only patients, for TB classification

But it did not perform so well on the DMTB patients

The type 1 interferon effect



From Orme, Robinson and Cooper, Nature Immunology 2015

Summary

To summarise, we can develop gene expression based algorithms to be used as diagnostic tools for TB and to measure TB treatment-response

But these need to take into account other factors and morbidities, such as

- diabetes
- HIV
- other co-infection – e.g. helminths
- Age?
- greater genetic variability
- Impact of medications

Can this methodology be employed for monitoring latent infection and treatment efficacy?

Acknowledgements

LSHTM
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Bachti Alisjabhana

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Jorge Coronel

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