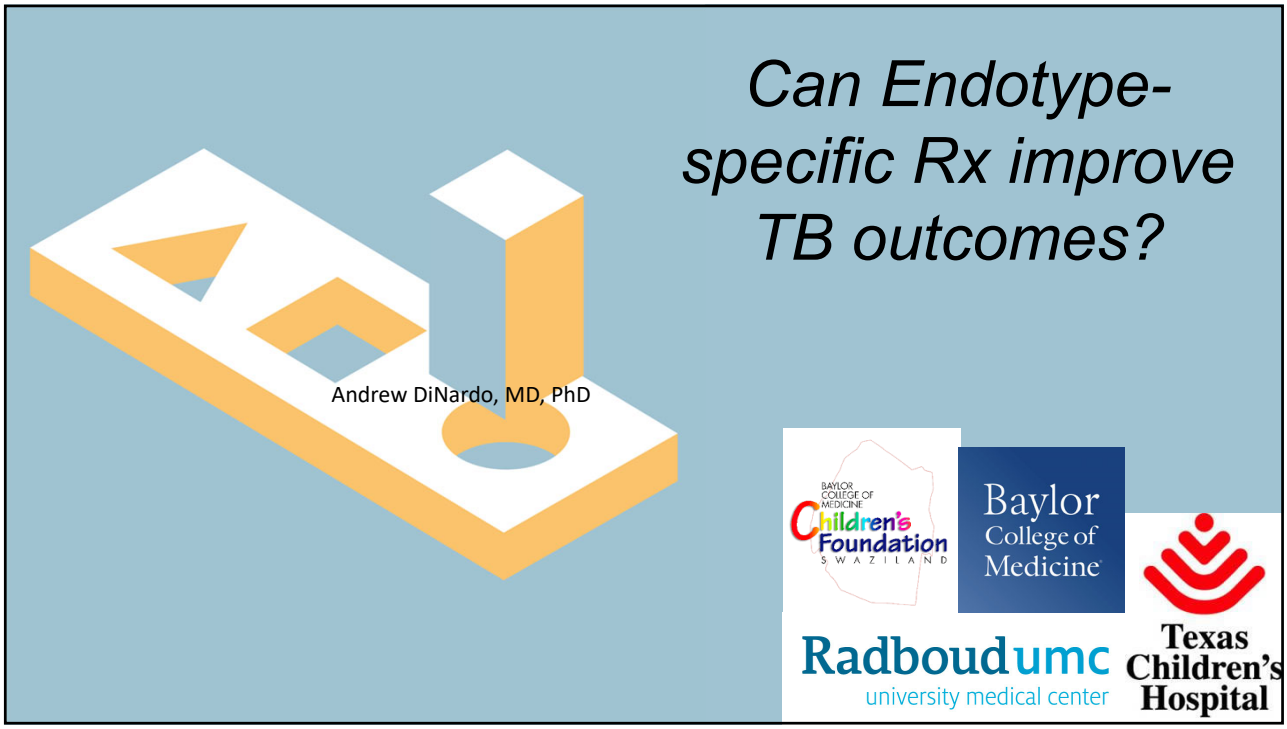


Endotypes:

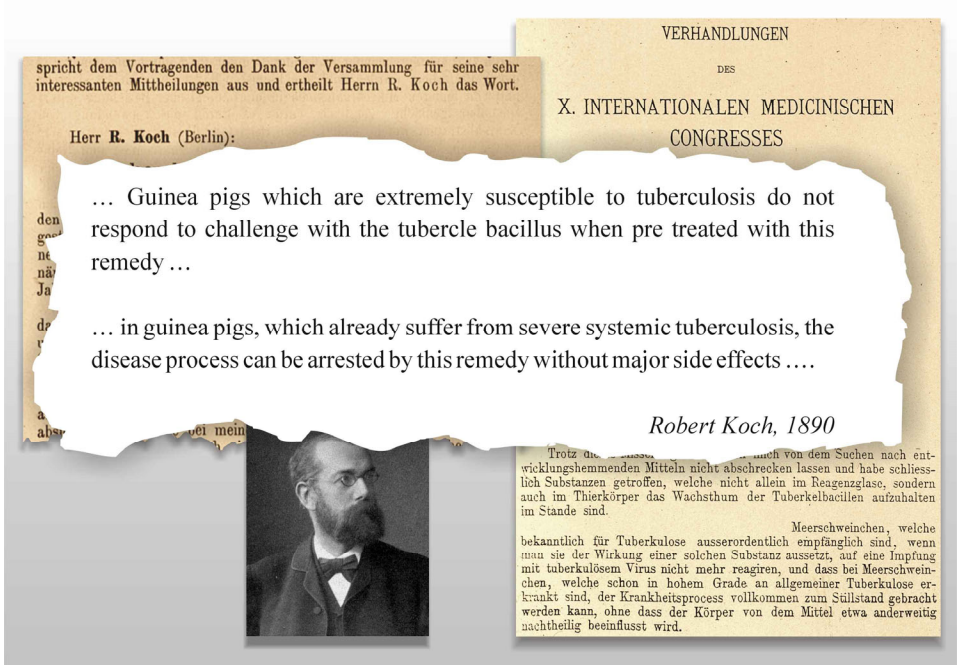
Distinct molecular perturbations

that lead to similar clinical phenotypes

and disease presentation

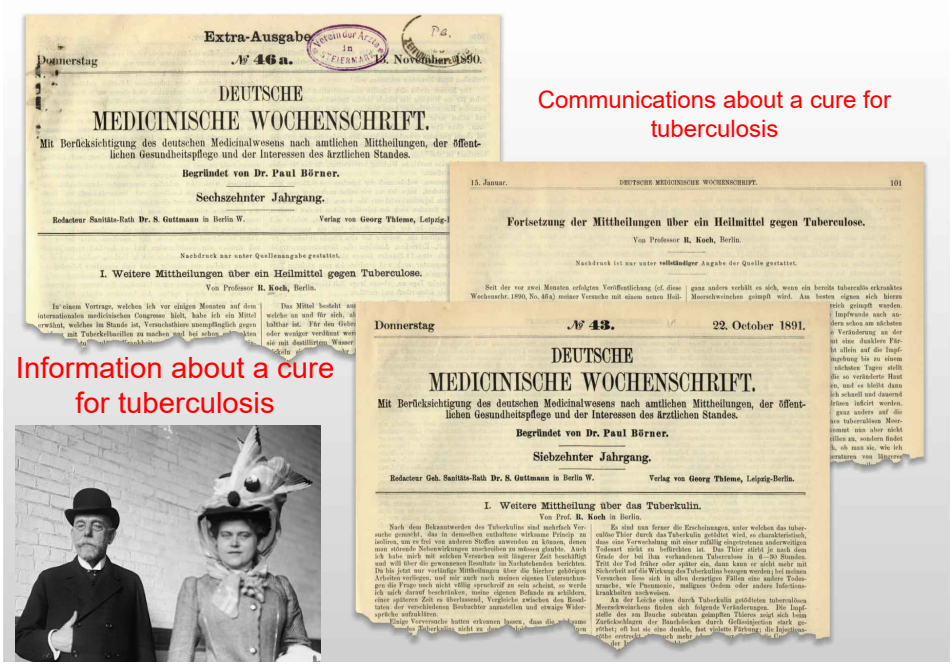
1882- 1891:

the historic Koch-Virchow skirmish



Max-Planck-Institut für Infektionsbiologie

Slide provided by S. Kaufmann



Slide adapted from S. Kaufmann

NEJM 1891

VIRCHOW ON THE EFFECTS OF KOCH'S METHOD.[1]

Professor Virchow illustrated the irritating effects of the fluid by the specimen of a brain removed from a child with tuberculous arachnitis, who died after four injections of the lymph amounting in all to two milligrammes. There was intense hyperæmia of the brain and pia mater such as Professor Virchow had never before seen. The vessels of the pia were extremely engorged and the brain-substance internally was of a dusky-red tint. The speaker could

1891:
An early
HDT failure

Tuberculin→ fatal immune reconstitution

by the injections of the lymph.

The *most important effect observed, however, was an eruption of fresh crops of tubercles after the injections.* This occurs especially in the pleura, pericardium, and peritoneum, and Virchow says that in the case of

body by infection with the products of disintegration. Virchow, therefore, urges the greatest caution in the use of the remedy. While admitting that in many cases the lymph does produce the effects claimed for it, he points out that this result is not constant, and he cites cases in which large masses of tubercle

out the whole extent of the larynx and trachea.

On January 14th, before the Berlin Medical Association, Professor Virchow resumed his lecture on the subject of cases which have resulted fatally after the inoculations of the Koch remedy. He said that he was not prejudiced against the remedy; he simply wished to give warning regarding its too general application. In the discussion which followed, Professors Frænkel and Baginsky spoke in support of Professor Virchow's contention that tubercular disease

1891 lessons:

1. Tuberculin induced fatal IRIS
2. One size doesn't fit all
3. TB M&M is not only due to bacilli

NEJM 1891

Problem #1: no single immune correlate of protection

What is "protective" in vitro can be fatal in vivo

IFN-γ improves in vitro *Mtb* killing
Recruits additional immune cells

1. ↑P-L maturation
2. Warburg metabolism
3. ROS upregulation
4. Ag processing
5. Chemokine activation

→ IFN-γ mediates killing of intracellular pathogens
and
→ Attracts additional immune cells

INFECTION AND IMMUNITY, Aug. 1976, p. 337-344
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Vol. 14, No. 2
Printed in U.S.A.

Partial Characterization of a Factor Extracted from Sensitized Lymphocytes That Inhibits the Growth of *Mycobacterium tuberculosis* Within Macrophages In Vitro

R. TURCOTTE,* Y. DES ORMEAUX, AND A. G. BORDUAS
Centre de Recherche en Immunologie, Institut Armand-Frappier, C.P. 100, Laval-des-Rapides, Ville de Laval, Québec, Canada H7N 4Z3

Received for publication 4 March 1976

IDENTIFICATION OF INTERFERON-γ AS THE LYMPHOKINE THAT ACTIVATES HUMAN MACROPHAGE OXIDATIVE METABOLISM AND ANTIMICROBIAL ACTIVITY*

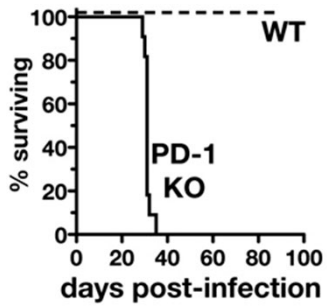
BY CARL F. NATHAN,‡ HENRY W. MURRAY,§ MICHAEL E. WIEBE, and BERISH Y. RUBIN

J. EXP. MED. © The Rockefeller University Press · 0022-1007/83/09/0670/20
Volume 158 September 1983 67

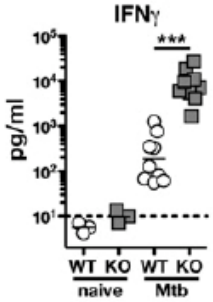
IFN-γ : fatal immune pathology

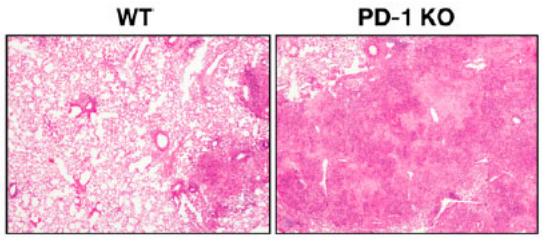
CD4 T Cells Promote Rather than Control Tuberculosis in the Absence of PD-1–Mediated Inhibition

Daniel L. Barber, Katrin D. Mayer-Barber, Carl G. Feng, Arlene H. Sharpe and Alan Sher



Inhibit PD1→↑IFNγ



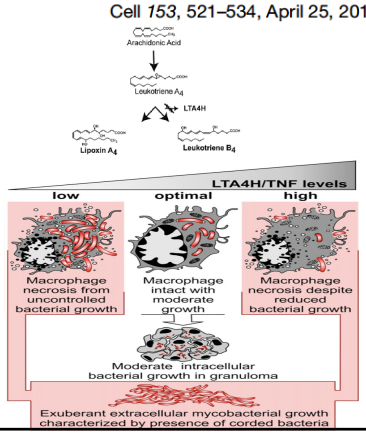


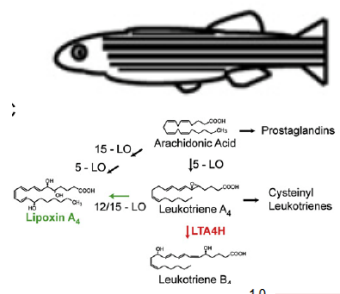
TNF narrow therapeutic window

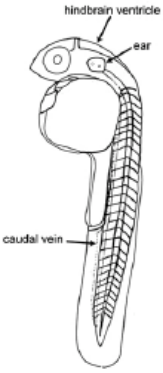
The *lta4h* Locus Modulates Susceptibility to Mycobacterial Infection in Zebrafish and Humans

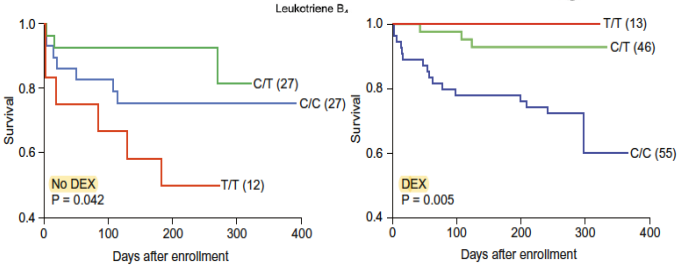
David M. Tobin,[1] Jay C. Vary, Jr.,[2,10] John P. Ray,[1,10] Gregory S. Walsh,[6] Sarah J. Dunstan,[6,7] Nguyen D. Bang,[6] Deanna A. Hagge,[8] Saraswoti Khadge,[9] Mary-Claire King,[2,3] Thomas R. Hawn,[2] Cecilia B. Moens,[5] and Lalita Ramakrishnan[1,2,4,*]

Cell *140*, 717–730, March 5, 2010
Cell *148*, 434–446, February 3, 2012
Cell *153*, 521–534, April 25, 2013





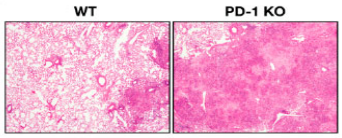




Immune induced pathology & death

CD4 T Cells Promote Rather than Control Tuberculosis in the Absence of PD-1–Mediated Inhibition

Daniel L. Barber,* Katrin D. Mayer-Barber,* Carl G. Feng,* Arlene H. Sharpe,† and Alan Sher*

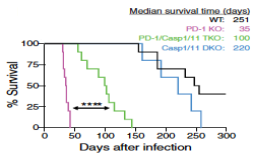


SCIENCE IMMUNOLOGY | RESEARCH ARTICLE

IMMUNOTHERAPY

PD-1 blockade exacerbates *Mycobacterium tuberculosis* infection in rhesus macaques

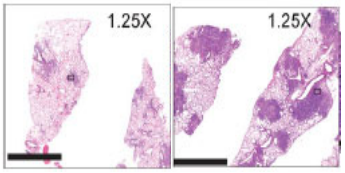
Keith D. Kauffman[1], Shunsuke Sakai[1], Nickiana E. Lora[1], Sivaranjani Namasivayam[2], Paul J. Baker[3], Olena Kamenyeva[4], Taylor W. Foreman[1], Christine E. Nelson[1], Deivide Oliveira-de-Souza[5], Caian L. Vinhaes[5], Ziv Yaniv[6], Cecilia S. Lindestam Arlehamn[7], Alessandro Sette[7,8], Gordon J. Freeman[9], Rashida Moore[10], NIAID/DIR Tuberculosis Imaging Program*, Alan Sher[2], Katrin D. Mayer-Barber[3], Bruno B. Andrade[5], Juraj Kabat[4], Laura E. Via[11]*, Daniel L. Barber[11]



BRIEF DEFINITIVE REPORT

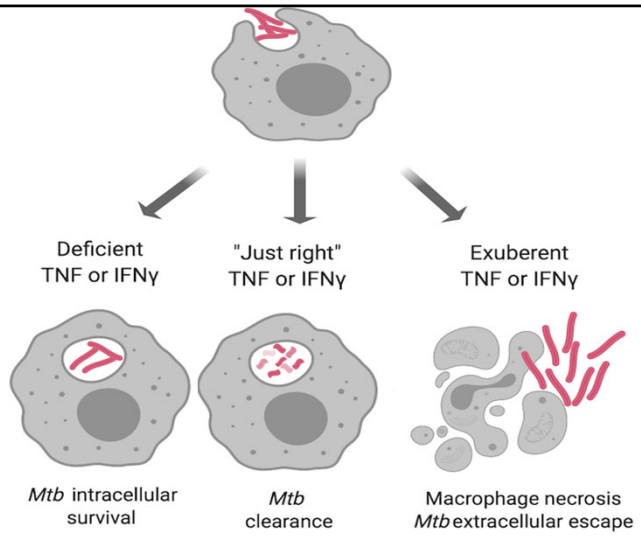
Irg1 expression in myeloid cells prevents immunopathology during *M. tuberculosis* infection

Sharmila Nair[1*], Jeremy P. Huynh[2*], Vicky Lampropoulou[3], Ekaterina Loginicheva[3], Ekaterina Esaulova[3,4], Anshu P. Gounder[2], Adrianus C.M. Boon[1,2,3], Elizabeth A. Schwarzkopf[3], Tara R. Bradstreet[3], Brian T. Edelson[3], Maxim N. Artyomov[3], Christina L. Stallings[2], and Michael S. Diamond[1,2,3,5]



Clinical trial of aerosolized IFN-γ in MDR TB halted early due to increased death (10 vs 5 deaths)

Dawson R, Condos R, Tse D, et al. Immunomodulation with recombinant IFN-γ in pulmonary tuberculosis. PLoS One 2009; 4:e6984.



Different (and contradictory) molecular endotypes can lead to similar clinical phenotypes

Endotypes

Distinct molecular perturbations that lead to similar clinical phenotypes and disease presentation

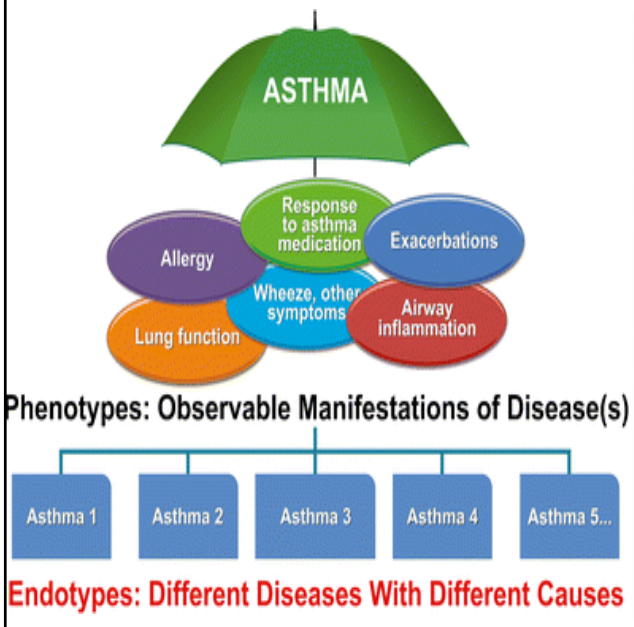
Putative Candidate Immune Endotypes	Immune Functional	*Mtb* killing capacity	Potential Clinical Implication	Literature supporting Hypothesis
IFN-γ Upstream Defects (IL12, IL12RB2, IKKB, IRF8)	Decreased IFN-γ production	Decreased	Augment ATT with exogenous IFN-γ	Bustamante 2014
IFN-γ Downstream Defects	Increased IFN-γ but decreased CXCL10, GBP and NOX downstream of IFN-γ	Decreased	Depends on mechanism of defect	Bustamante 2014
IFN-γ Auto-antibodies (IFN-γ Downstream Defect)		Decreased	Rituximab to Rx anti-IFN-γ auto-antibodies.	
Exuberant IFN-γ	Excessive collateral lung damage, lung necrosis, escape of viable Mtb into extracellular space	Increased *in vitro*; Decreased *in vivo*	Anti-IFN-γ neutralizing antibodies	Sekai 2016
TNF deficiency	Loss of TNF downstream intracellular phago-lysosome maturation	Decreased	Exogenous TNF; Depends on why TNF is deficient	Ehlers 2003
Exuberant TNF	Macrophage necroptosis before killing of intracellular Mtb; Mtb escapes to extracellular space	Decreased	TNF inhibitor	Tobin 2012; Roca 2013
Excess immune checkpoint inhibitor	Decreased IL-2, TNF, IFN-γ	Decreased	Immune Checkpoint inhibitors	Singh 2013
Deficient Immune Checkpoint inhibitors	Excessive IL-2, TNF, IFN-γ results in pathologic immunity, collateral lung damage and viable Mtb grows in extracellular space	Increased in vitro; decreased in vivo	Immunosupressants	Barber 2012 & Sakai 2016
GM-CSF deficiency	Leads to IFN-γ and TNF deficiency. See notes above	Decreased	Exogenous GM-CSF. Rituximab to Rx anti-GM-CSF autoantibodies	Rosen & Holland 2013
None; immune system is intact; individual exposed to high Mtb inoculum or hyper-virulent Mtb strain	Functional immunity;	Normal/ increased		Howard 2019;

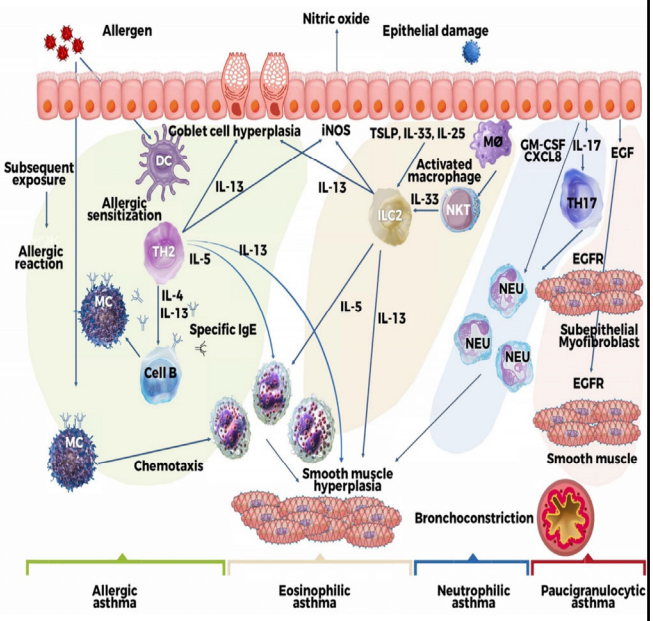
Existing Endotypes

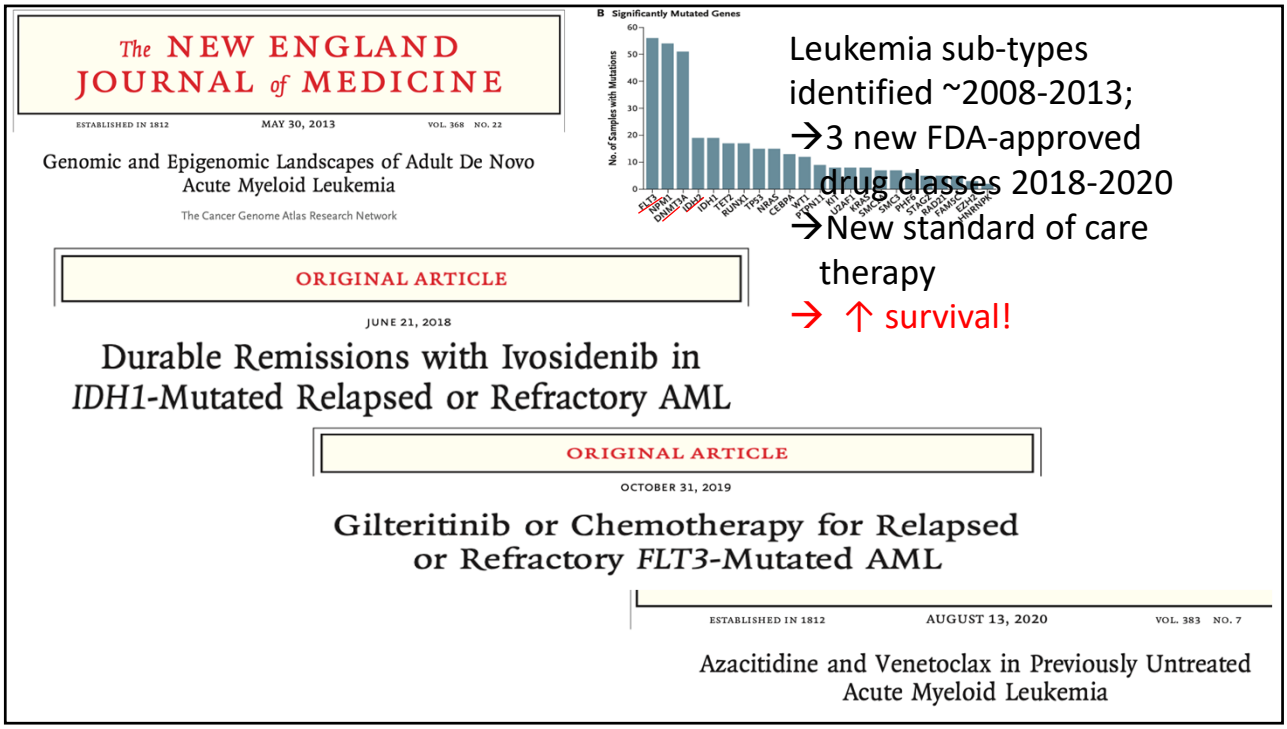
- Distinct molecular perturbations that lead to similar clinical phenotypes and disease presentation
- Examples
  - CLL **
  - AML **
  - Breast Cancer **
  - Asthma*
  - Sepsis
  - Acute Respiratory Distress Syndrome

*Endotype-specific treatment is Standard of care

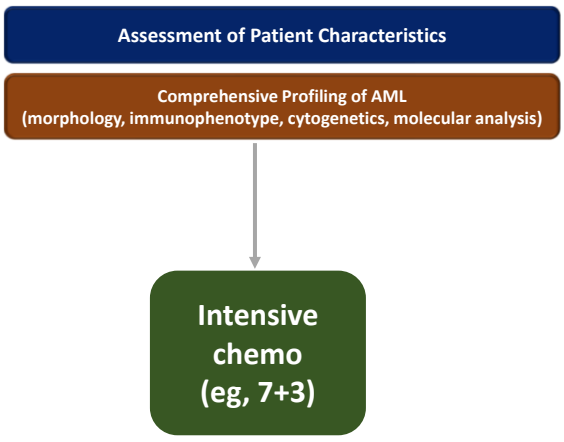
* * Endotype-specific treatment improved mortality



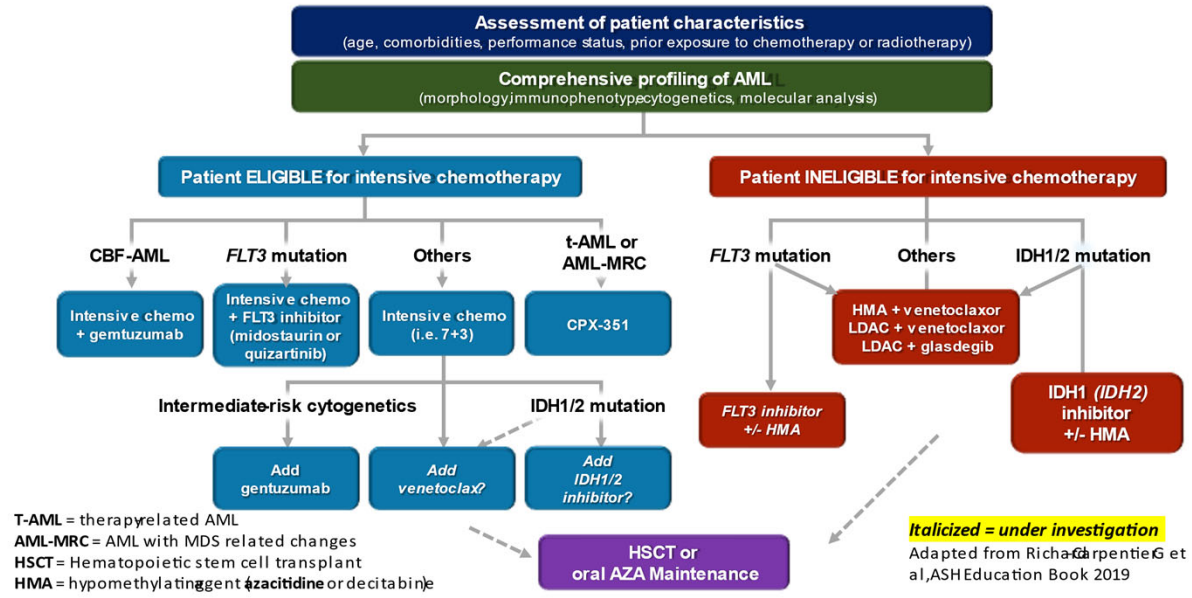




Pre- endotype identification Leukemia Rx (2017)



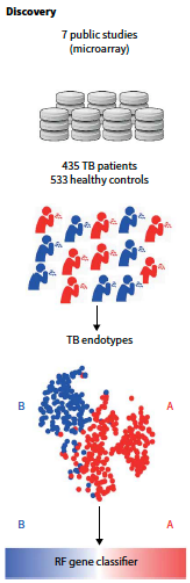
Post endotype identification Leukemia Rx (2024)



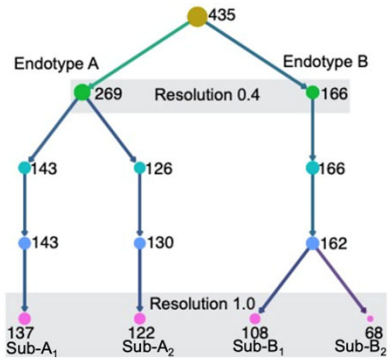
Unbiased approach:

Preliminary evidence for TB endotypes

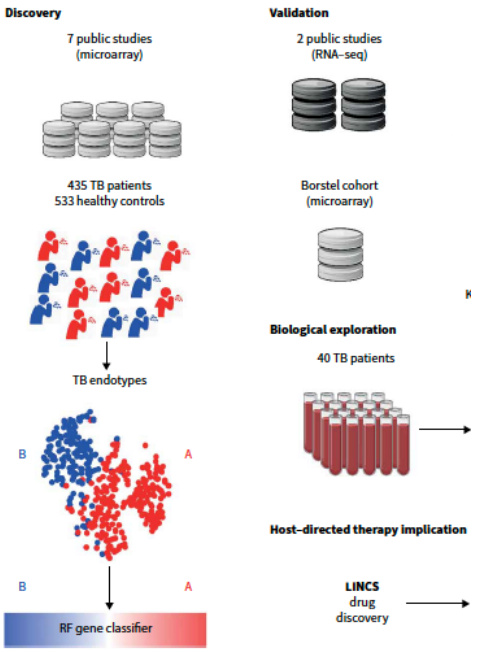
Evidence for TB endotypes:



- Data mined 9 TB studies from 7 different countries
- 435 TB cases & 533 healthy controls
- Unbiased Seurat clustering
- 2-4 different TB endotypes identified

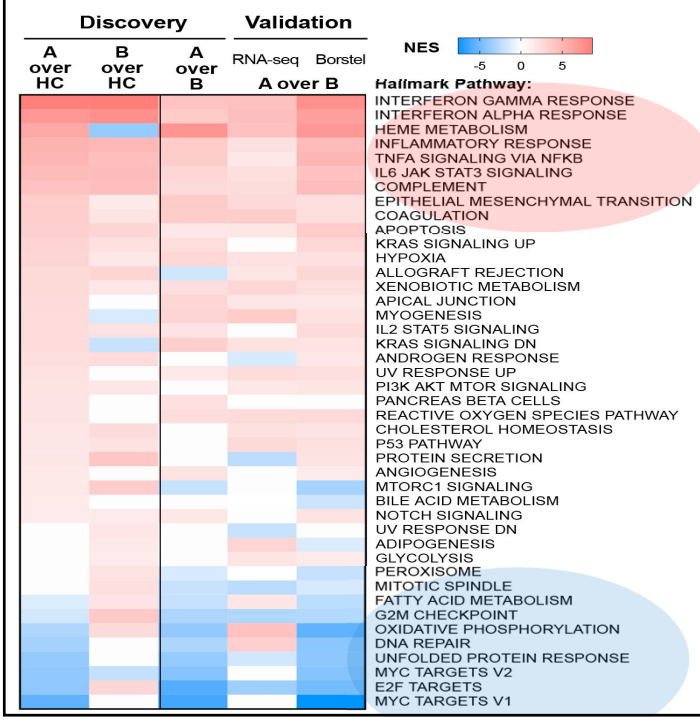


ERJ 2022



- Validated w 2 unique RNAseq datasets
- Validated with German/ Romanian cohort
- Validated with Eswatini immunology cohort
- Linked to data base of HDT drugs

ERJ 2022



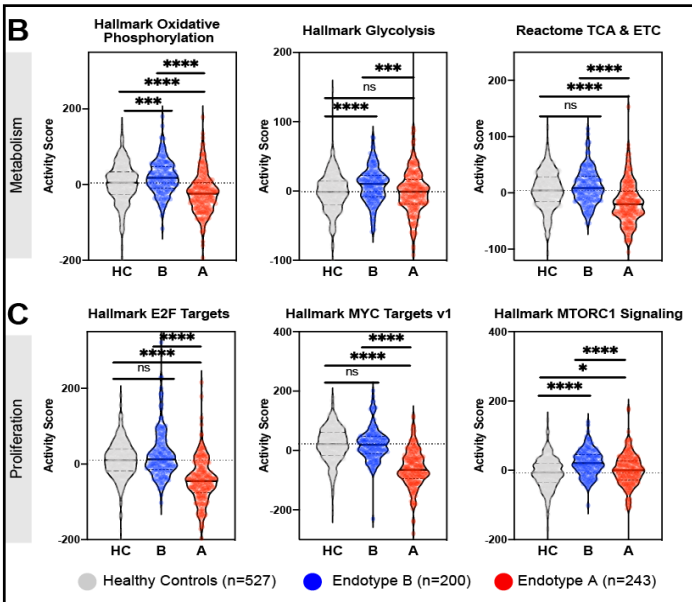
Endotype A:

- Enriches for IFN, Heme, inflammatory, TNF, IL6

Endotype B

- Metabolic and proliferative pathways

ERJ 2022



Endotype A:

- Enriches for IFN, Heme, inflammatory, TNF, IL6

Endotype B

- Metabolic and proliferative pathways

ERJ 2022

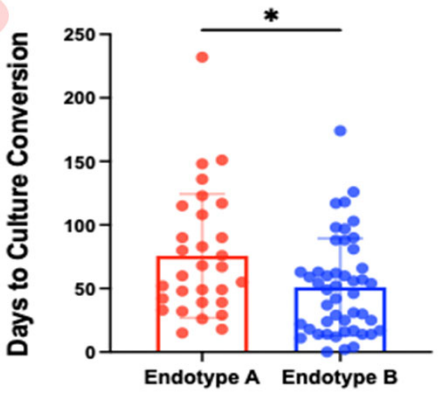
Table 1. Epidemiologic characteristics of the German and Romanian (Borstel) validation cohort.

	All	Endotype A	Endotype B	p-value*
Cavitary Disease	71.7% (66/92)	76.3% (29/39)	68.5% (37/54)	0.485
MDR TB	61.9% (75/121)	44% (22/50)	74.6% (53/71)	0.001
Baseline TTP (days)	17	15	17.5	0.443
Current or previous smoker	53.2% (33/62)	41.1% (7/17)	57.7% (26/45)	0.268
Median BMI	20.7	18.8	21.2	0.144
% Male	62.2% (56/90)	65.7% (23/35)	60.0% (33/55)	0.658
Median age	37.9	42.9	37.6	0.253
Median TCC (days)	54	63.5	49	0.017
Favorable outcome	80.9% (51/63)	70.5% (24/34)	93.1% (27/29)	0.027
Mortality	6.3% (4/63)	11.7% (4/34)	0% (0/29)	0.117

MDR, multi-drug resistance; TTP, time to positivity; BMI, body mass index; TCC, time to culture conversion. (Not all data was available for all participants).
**Mann-Whitney U test or Fisher's exact test were used to determine significance, as appropriate.*

Endotype A:

- Slower time to culture conversion
- Less favorable outcomes



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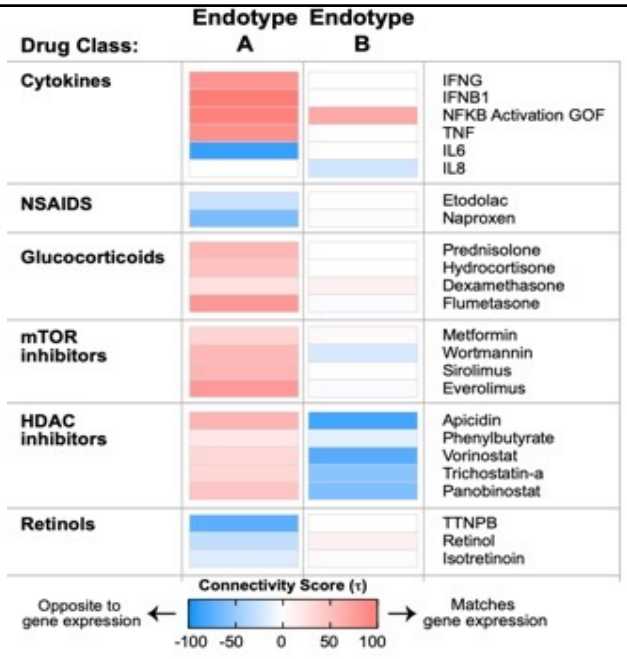
LINCS: in silico HDT drug discovery

HDT beneficial for one endotype either inconsequential or detrimental for other endotype



ERJ 2022

Transcriptomics is not clinically relevant.

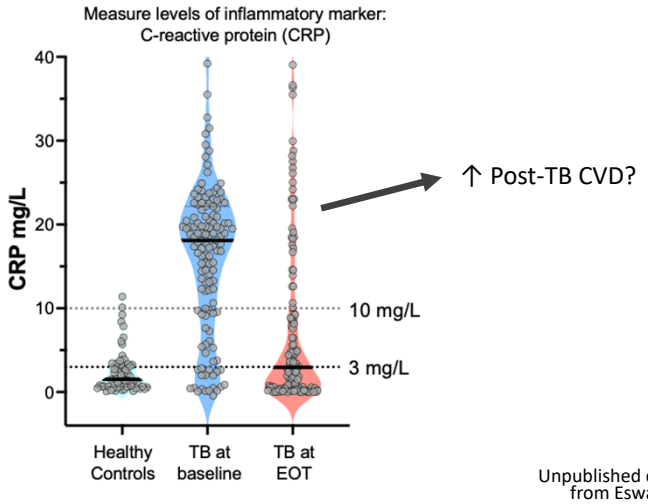
How can host immunity and inflammation be assessed now?

Non-evidence based!

Assessing Inflammation & Immune responsive now

Inflammation

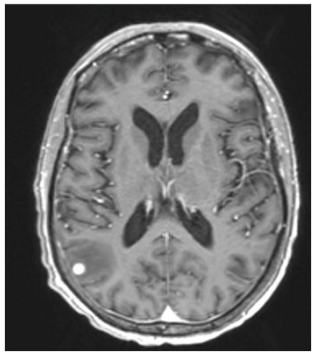
- CRP, ESR



Unpublished data from Eswatini

Assessing Inflammation & Immune responsive now

Inflammation
- CRP, ESR
- Cytokine panel*

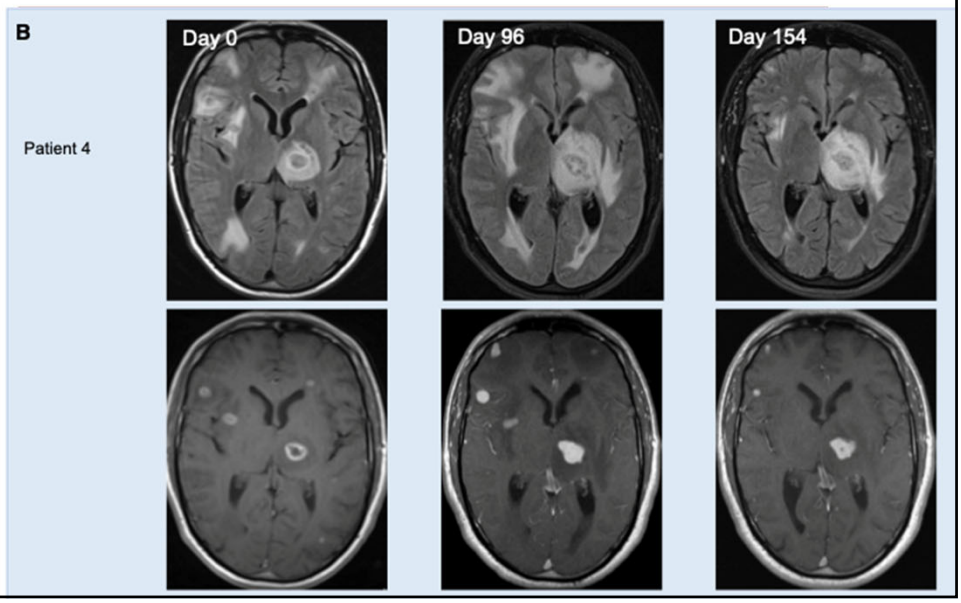


ARUP 13 cytokine panel; $150 USD

Components	
Interleukin 2 Receptor, Soluble, Serum	→ Dexamethasone
Interleukin 12, Serum	
Interferon gamma, Serum	
Interleukin 4, Serum	
Interleukin 5, Serum	
Interleukin 10, Serum	
Interleukin 13, Serum	
Interleukin 1 beta, Serum	→ Anakinra
Interleukin 6, Serum	→ Tocilizumab
Interleukin 8, Serum	
Tumor Necrosis Factor - alpha, Serum	→ Infliximab
Interleukin 2, Serum	
Interleukin 17, Serum	

Case Report

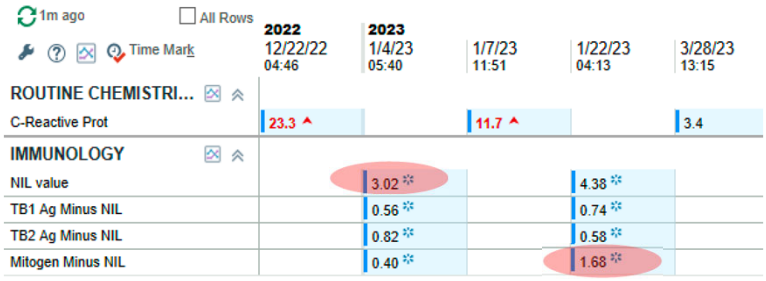
Interleukin-1 receptor antagonist anakinra as treatment for paradoxical responses in HIV-negative tuberculosis patients: A case series



Assessing Inflammation & Immune responsive now

Inflammation
- CRP, ESR
- Cytokine panel

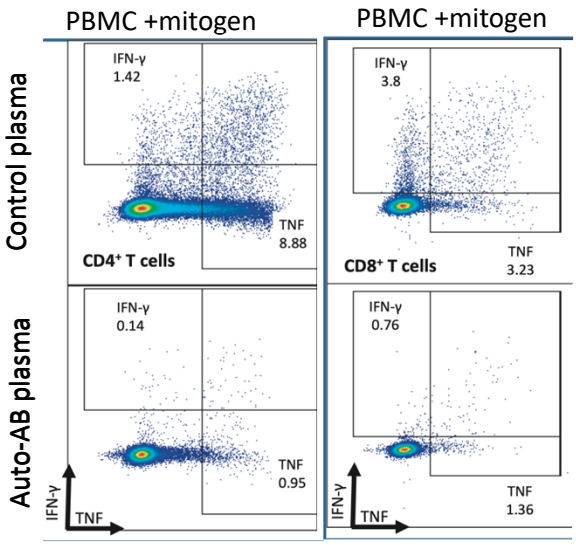
Immune Responsiveness
- QuantiFERON



Assessing Inflammation & Immune responsive now

Inflammation
- CRP, ESR
- Cytokine panel

Immune Responsiveness
- QuantiFERON
- Fxnal Flow Cytometry*



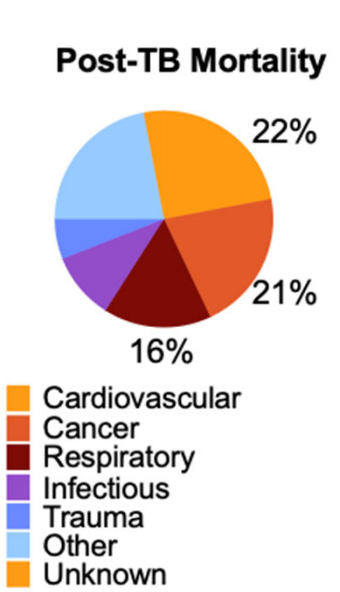
But these are super extreme cases.

How does this apply to “regular” TB cases?

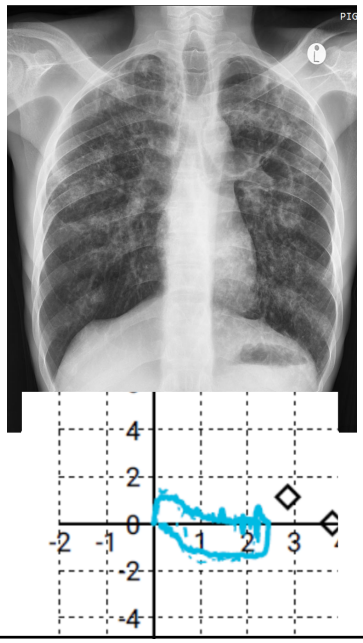
Is TB curable with antibiotics?

Microbiologic cure ≠ health

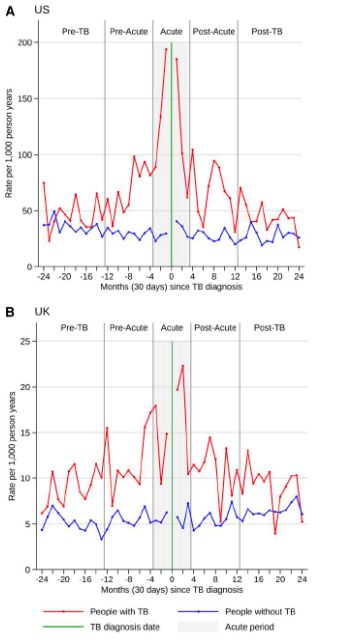
3.7-fold Risk Death post-TB



Romanowski 2019



CVD incidence & TB



Critchley 2024

Baseline

Test1 2024-01-09 10:28

Parameter	Best	LLN	Z-sc.	%Pred
FVC[L]	4.76	3.22	1.21	115.8
FEV1[L]	3.59	2.62	0.40	105.6
FEV1/FVC	0.75	0.73	-1.17	
FEF2575[L/s]	3.68	1.90		104.9
PEF[L/s]	6.96	--		--
FET[s]	10.99			
FIVC[L]	4.11	3.22		100.1
PIF[L/s]	5.01	--		--

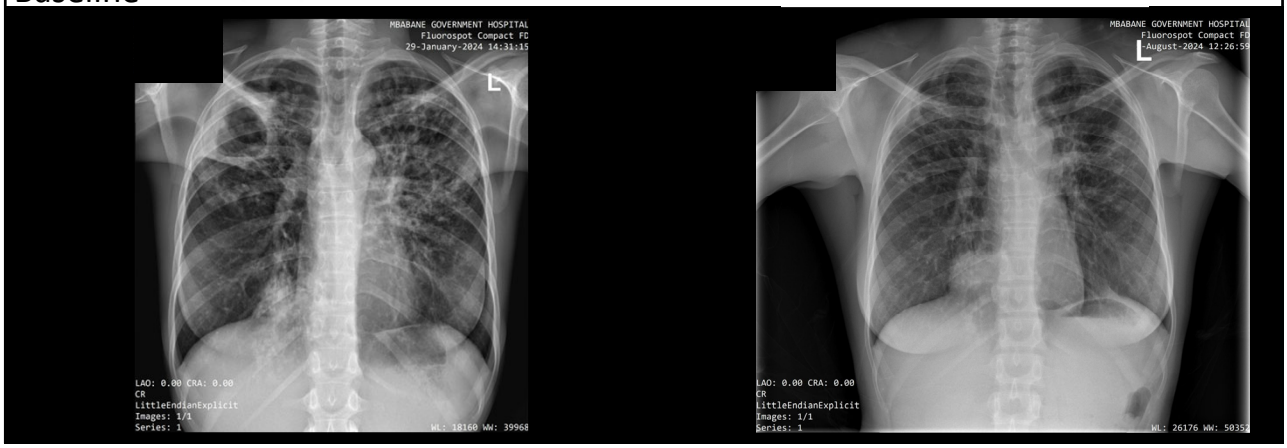
FEV1 Var = 247mL 6.9%, FVC Var = 774mL 16.3%
Test quality FEV1 - D, FVC - E
Normal spirometry

EOT

Test1 2024-07-16 10:45

Parameter	Best	LLN	Z-sc.	%Pred
FVC[L]	4.26	3.21	0.30	104.0
FEV1[L]	3.67	2.61	0.59	108.3
FEV1/FVC	0.86	0.72	0.56	
FEF2575[L/s]	4.46	1.88		127.6
PEF[L/s]	6.71	--		--
FET[s]	6.64			
FIVC[L]	4.13	3.21		100.7
PIF[L/s]	5.56	--		--

FEV1 Var = 48mL 1.3%, FVC Var = 129mL 3.0%
Test quality FEV1 - A, FVC - A
Normal spirometry



Baseline

Parameter	Best	LLN	Z-sc.	%Pred
FVC[L]	4.83	3.00	1.52	122.2
FEV1[L]	2.07	2.32	-2.15	66.0
FEV1/FVC	0.43	0.69	-5.72	
FEF2575[L/s]	0.46	1.12		15.4
PEF[L/s]	4.31	4.88		57.0
FET[s]	16.31			
FIVC[L]	3.84	3.00		97.2
PIF[L/s]	3.91	--		--

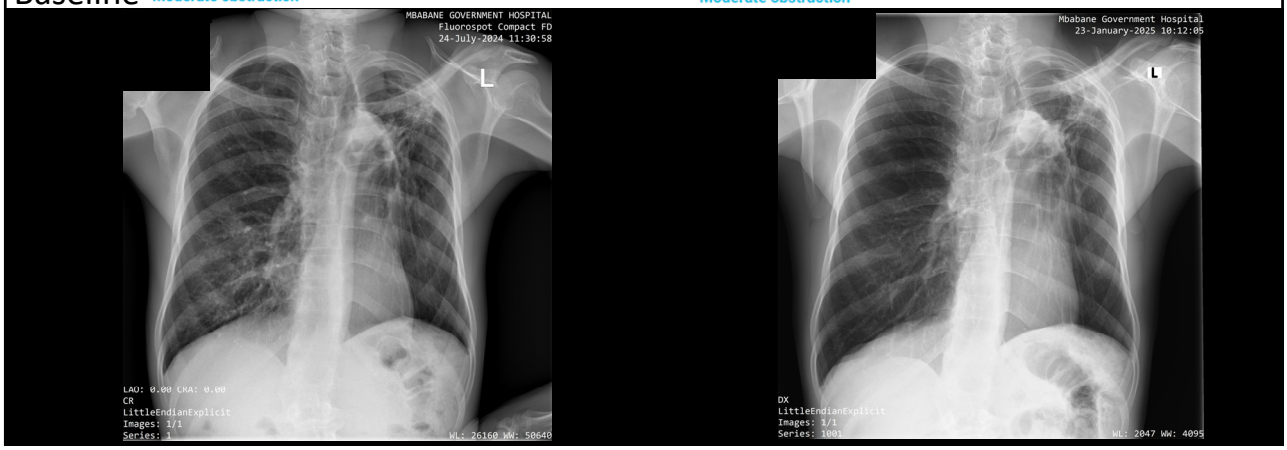
FEV1 Var = 34mL 1.6%, FVC Var = 503mL 10.4%
Test quality FEV1 - A, FVC - E
Moderate obstruction

EOT

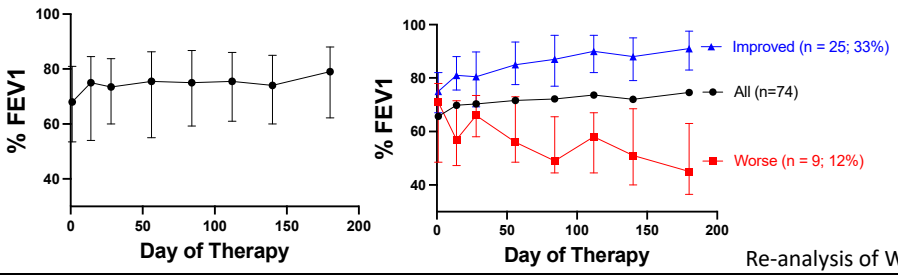
Parameter	Best	LLN	Z-sc.	%Pred
FVC[L]	5.31	2.92	3.38	143.4
FEV1[L]	2.32	2.24	-1.48	78.1
FEV1/FVC	0.44	0.67	-5.09	
FEF2575[L/s]	0.26	1.90		8.0
PEF[L/s]	5.27	6.01		70.4
FET[s]	16.22			
FIVC[L]	4.10	2.92		110.7
PIF[L/s]	3.64	--		--

FEV1 Var = 81mL 3.5%, FVC Var = 821mL 15.5%
Test quality FEV1 - A, FVC - E
Moderate obstruction

No improvement

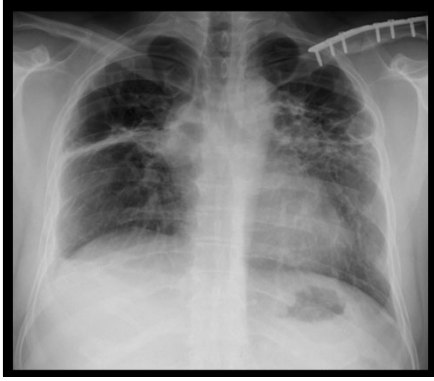


"paradoxical" worsening

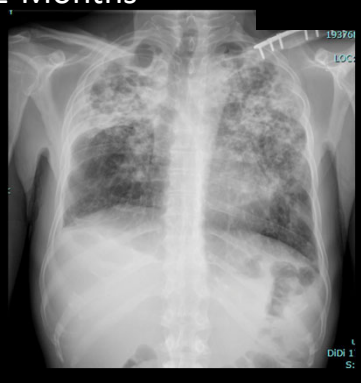


Re-analysis of Wallis 2021

Baseline



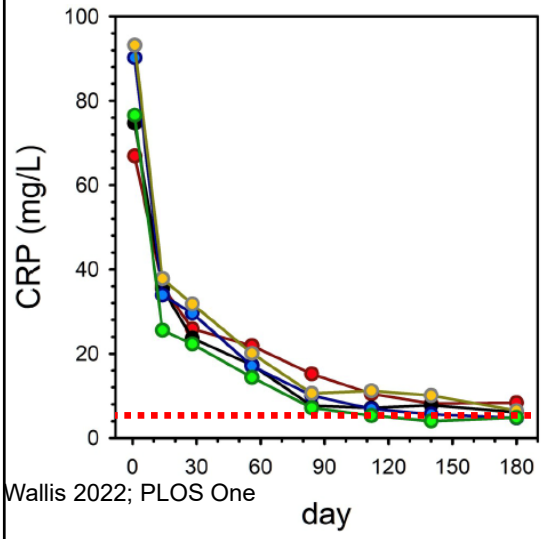
2-Months



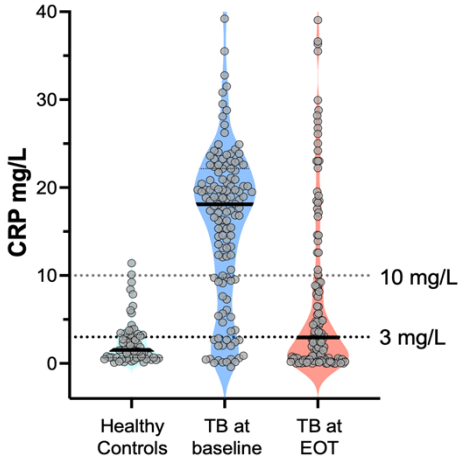
Problem #2: Antibiotics don't resolve inflammation

Problem #3: Antibiotics don't resolve anergy

~half TB survivors remain hyper-inflammatory



Wallis 2022; PLOS One

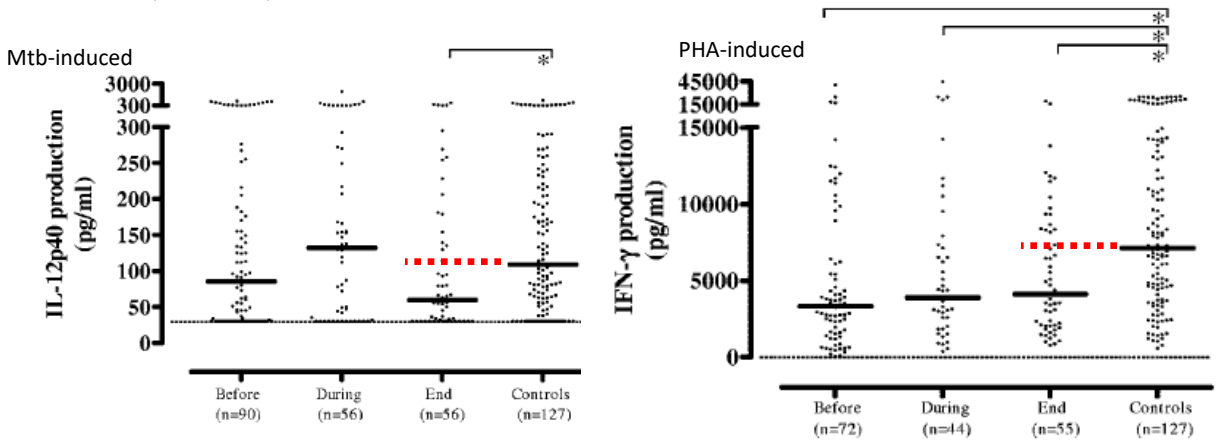


Unpublished Eswatini

CRP > 3mg/L Increased Risk CVD

Dynamic Changes in Pro- and Anti-Inflammatory Cytokine Profiles and Gamma Interferon Receptor Signaling Integrity Correlate with Tuberculosis Disease Activity and Response to Curative Treatment[▽]

Edhyana Sahiratmadja,[1,2] Bachti Alisjahbana,[3] Tjitske de Boer,[1] Iskandar Adnan,[2] Anugrah Maya,[4] Halim Danusantoso,[4] Ronald H. H. Nelwan,[3] Sangkot Marzuki,[2] Jos W. M. van der Meer,[6] Reinout van Crevel,[6] Esther van de Vosse,[7] and Tom H. M. Ottenhoff[1]*



~half TB survivors remain hypo-responsive

Summary & unanswered questions

- Multiple TB endotypes
- Therapy often should include immune suppressive or immune activating HDT

What is the best way to identify:

- TB endotype suitable for only 2 or 4 mo of antibiotics?
- TB endotype at risk post-TB CVD?
- TB endotype at risk for PTLD?