Biomarker study approaches

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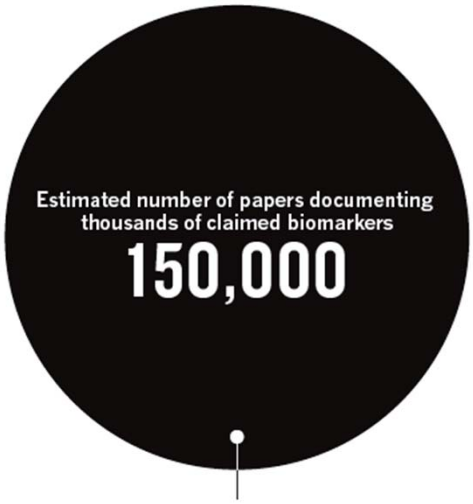
satvi CIDRI CLINICAL INFECTIOUS DISEASES RESEARCH INITIATIVE IIDMM INSTITUTE OF INFECTIOUS DISEASE AND MOLECULAR MEDICINE

A DROP IN THE OCEAN

Few of the numerous biomarkers so far discovered have made it to the clinic.



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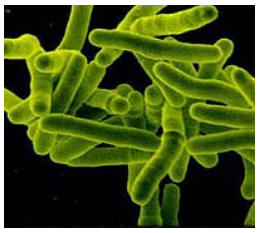
G Poste. Nature 2011;459:156

Biomarker for what?



1. Correlate of protection against infection
2. Correlate of protection against disease
3. Correlate of risk of infection
4. Correlate of risk of disease
5. Correlate of infection
6. Correlate of disease
7. Correlate of disease severity
8. Correlate of prognosis
9. Correlate of drug resistance
10. Correlate of response to therapy
11. Correlate of relapse following therapy

1. Aim

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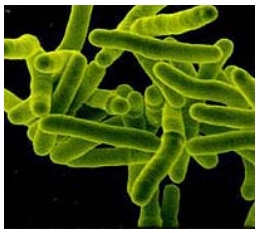
Biomarkers for TB vaccinology



- Correlate of risk of disease
  Targeted populations in efficacy trials
- Correlate of protection against disease
  Quick screens of vaccine candidates

2. Rationale

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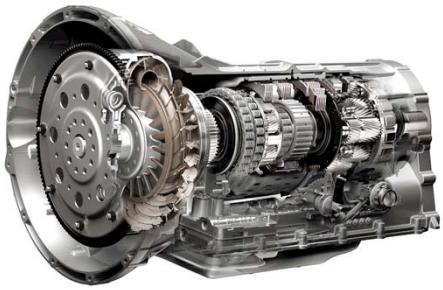
Is this important?



- 1.7 million die from TB each year
- Models: Vaccines that interrupt transmission will have major impact

3. Significance

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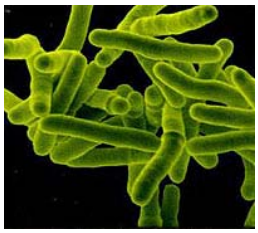
Is this novel?



- Validated biomarkers have not been identified to date, *using our approach*

4. Innovation

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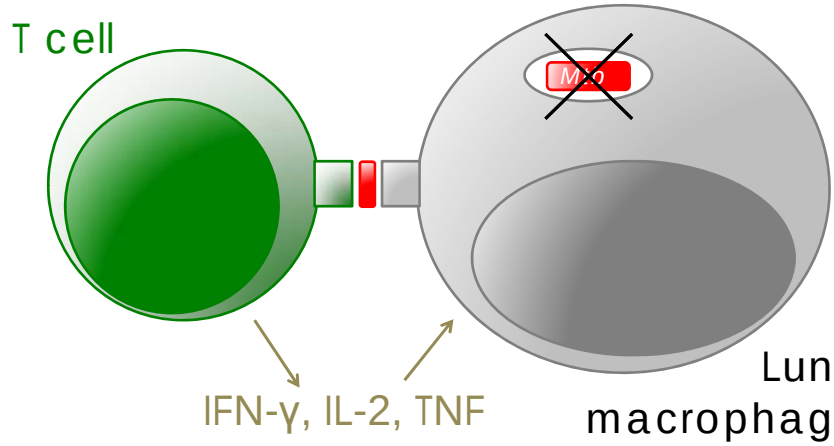
What do you expect to find?



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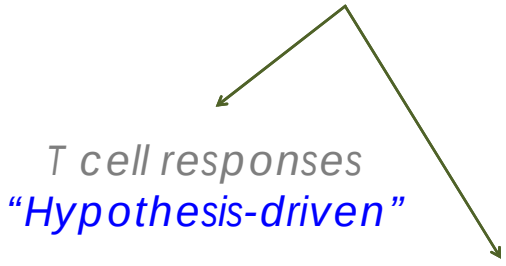
What do you expect to find?



T cell responses
"Hypothesis-driven"

Gene expression profiles
"Unbiased"

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"Unbiased" approaches

1. Transcriptomics
2. Proteomics
3. Metabolomics

etc.

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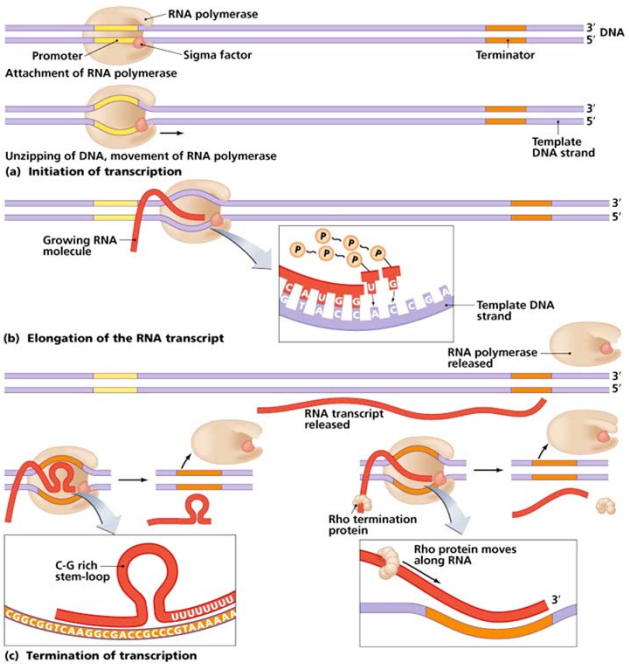
Transcriptomics: measure mRNA

Gene expression



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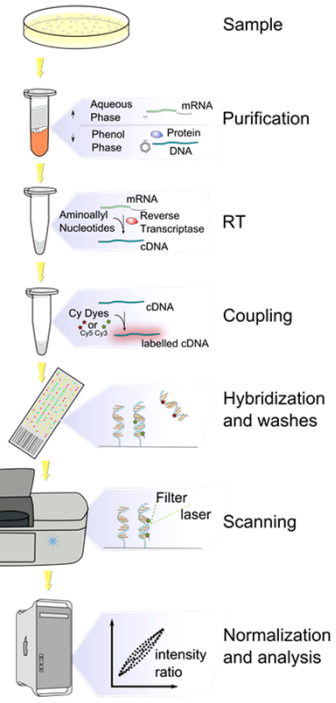
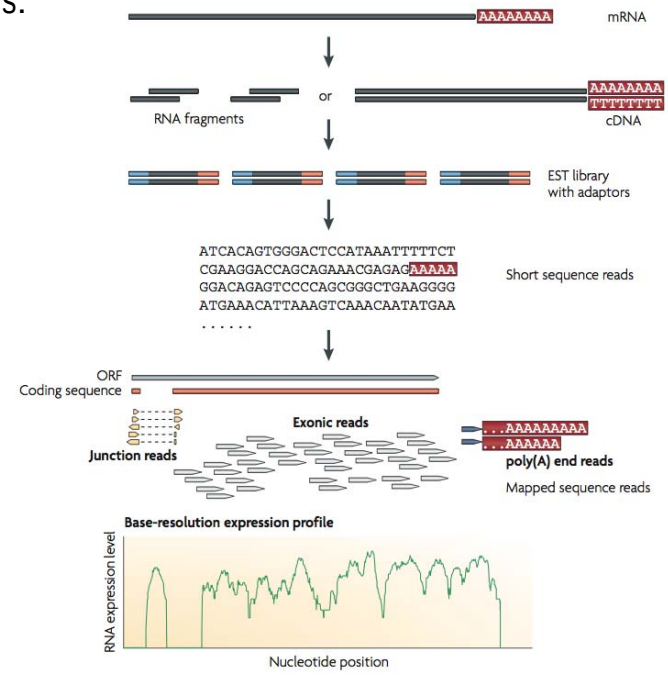
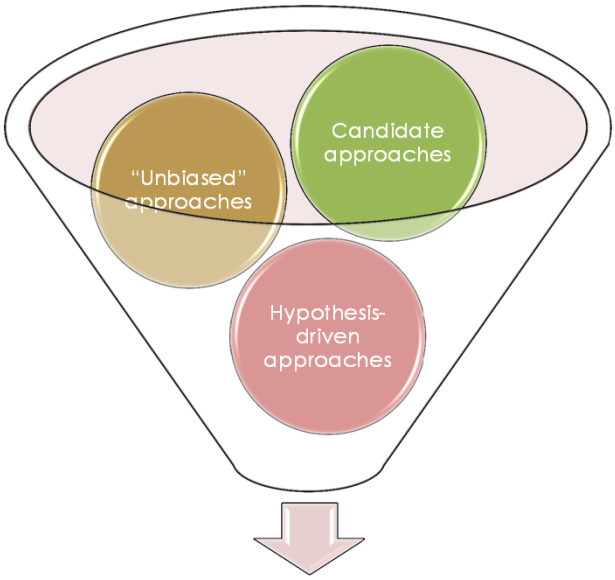
Transcriptomics: 1. DNA microarrays



Transcriptomics: 2. RNA-Seq





Biomarker

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How would you do a CoR study?

L Qin JID 2007;196:1304

6. Methods

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How would you do a CoP study?

L Qin JID 2007;196:1304

6. Methods

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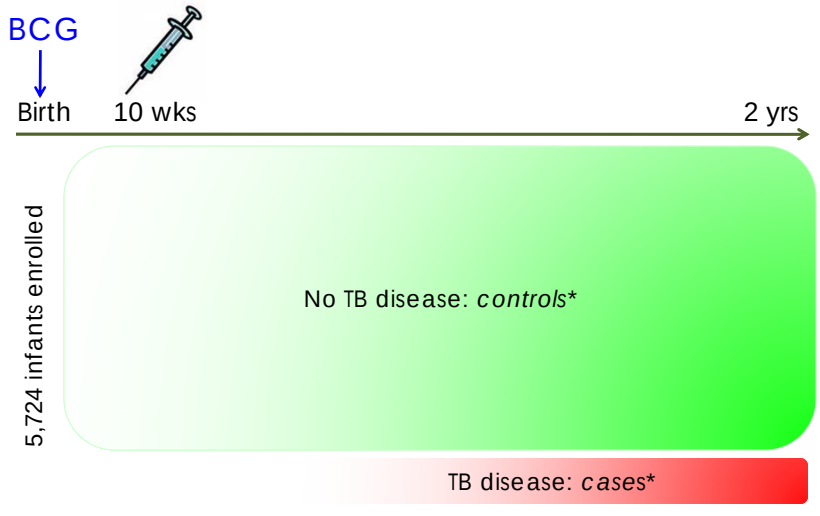
To determine correlates of risk of TB disease, following BCG vaccination



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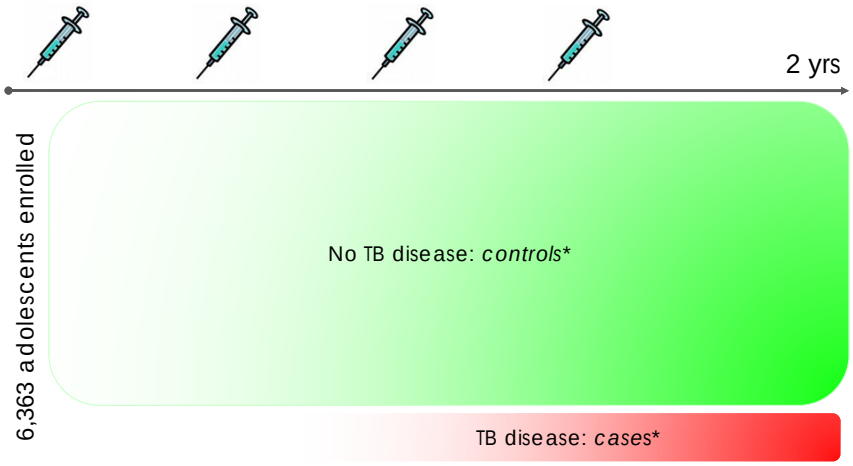
*Training and validation sets

To determine correlates of risk of TB disease, following infection with *Mtb*



*Training and validation sets

Tom Scriba, Adam Penn-Nicholson, Hassan Mahomed, Dan Zak, Alan Aderem, many others.

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To determine correlates of risk of TB disease, following infection with *Mtb*

Inclusion criteria

- QFT and/or TST+
- No TB for 6 months
- HIV-negative

Matching

- Cases randomly assigned to training and validation sets
- Cases were divided into groups (bins) according to the following variables (in order of importance):
    1. *Passive or active original study arm*
    2. *Previous episode of TB*
    3. *Age at enrollment*
    4. *Gender*
    5. *Ethnicity*
- Controls that matched all 5 variables were assigned to each case at a 2:1 ratio (for cross-sectional) or 1:1 ratio (for longitudinal)

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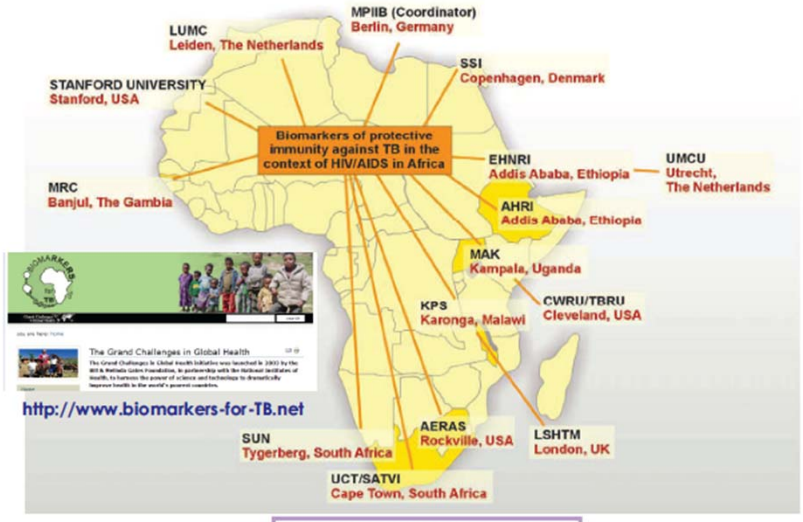
Slide courtesy of Tom Scriba

Grand Challenges in Global Health #6-74

Multiple study sites and partners



https://gc6.biomarkers-for-tb.net

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New case definitions

Category	Culture 1	Culture 2	AFB 1	AFB 2	CXR	Symptoms	TB treatment	Treatment response	Failed AB	Original Class	New Class
1	pos	pos	p/n	p/n	p/n	p/n	p/n	p/n	p/n	Definite	Definite
2	pos		p/n	p/n	pos	p/n	p/n	p/n	p/n	Definite	Definite
3	pos		pos	p/n		pos	p/n	p/n		Probable	Definite
9	pos		pos				p/n	p/n		Probable	Definite
4	pos					pos	p/n	pos		Probable	Probable
10	pos					pos	p/n			Probable	Probable
7			pos	pos	pos	p/n	p/n	p/n		Probable	Probable
8			pos	pos		pos	p/n	p/n		Probable	Probable
5			pos		pos	pos	p/n	p/n		Probable	Probable
6			pos		pos	p/n	p/n	pos		Probable	Probable
17			pos		pos		p/n			Questionable	Probable
14			pos			pos	p/n			Questionable	Possible
18			pos			pos	p/n			Questionable	Possible
11					pos	pos	p	pos	Pos	Possible	Possible
12					pos	pos	p	pos		Questionable	Questionable
16					pos	pos	p/n			Questionable	Questionable
13					pos		p	pos		Questionable	Questionable
15						pos	pos	pos		Questionable	Questionable
new					pos		p/n				Questionable
new	pos						p/n				Questionable
new			pos				p/n				Questionable
19						pos	pos	p/n		Questionable	Questionable
20						pos	Neg			Non-TB case	Non-TB case
21	ND	ND	ND	ND	ND	neg	neg			Non-TB case	Non-TB case

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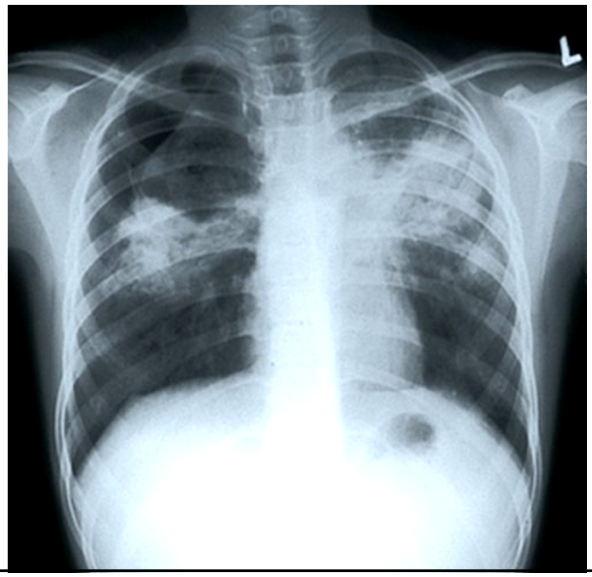
Which sample to use for analysis?



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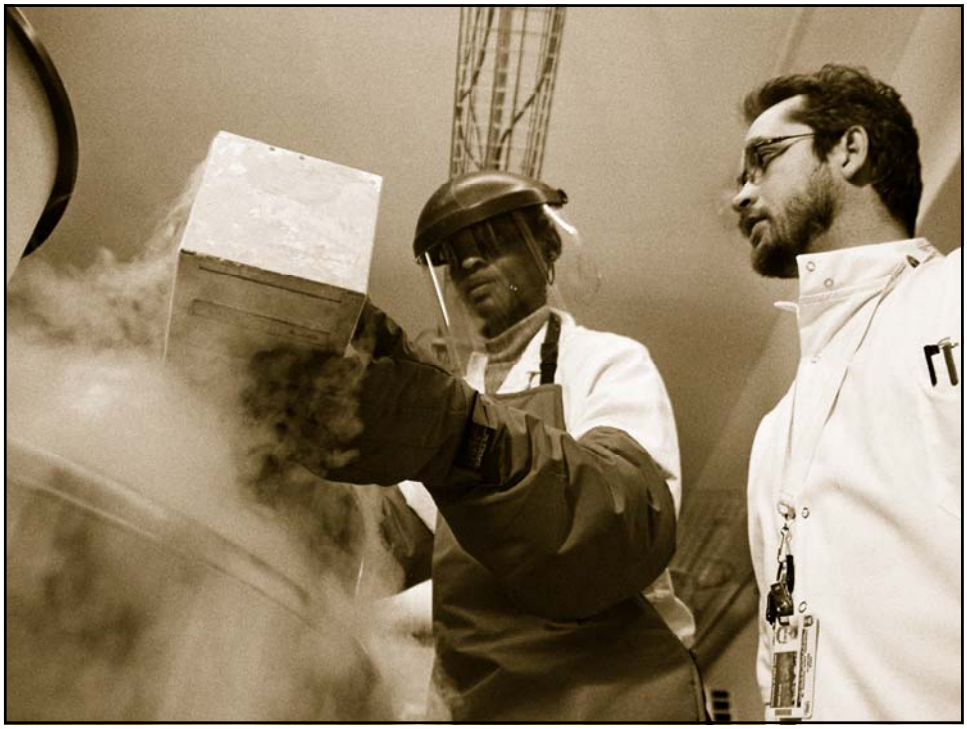

Specific gene expression analysis in the adolescent study

	PBMC	Monocytes
		Purify CD14+ Monocytes
Antigens	Ag85A/B	-
	ESAT-6/CFP-10	-
	H37Rv	H37Rv
	Unstimulated	Unstimulated
	↓	↓
	Purify T cells	↓
	RNA isolation, RNA-seq	RNA isolation, RNA-seq

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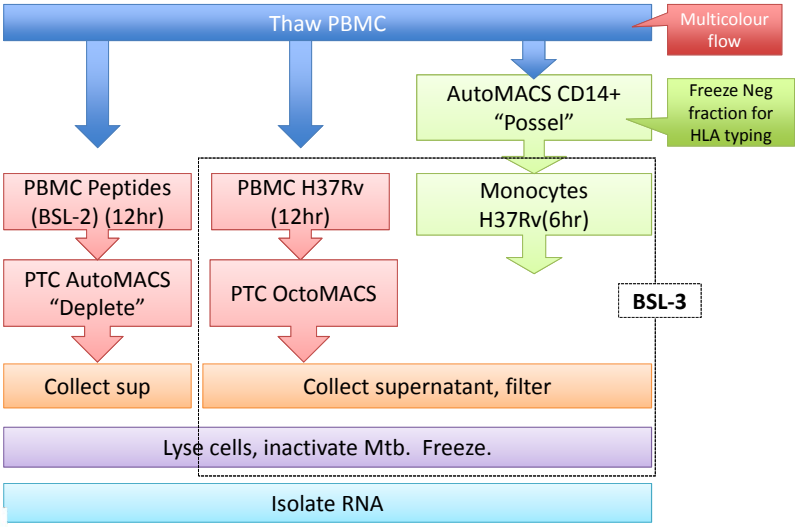
Slide courtesy of Adam Penn-Nicholson

Specific gene expression analysis in the adolescent study



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Bioinformatics approaches....1.

vs.

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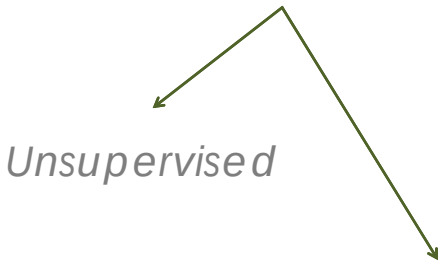
Bioinformatics approaches....2.



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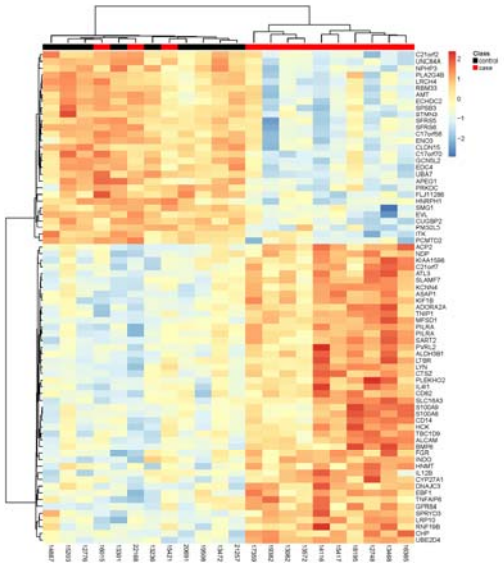
Gene expression signatures that associate with risk of TB, after *Mtb* infection



Helen Fletcher, Ali Filali, Rafick-Pierre Sekaly, many others.
*DNA microarray analysis of RNA from PBMC from 10 wks of age, incubated with BCG for 12 hours.

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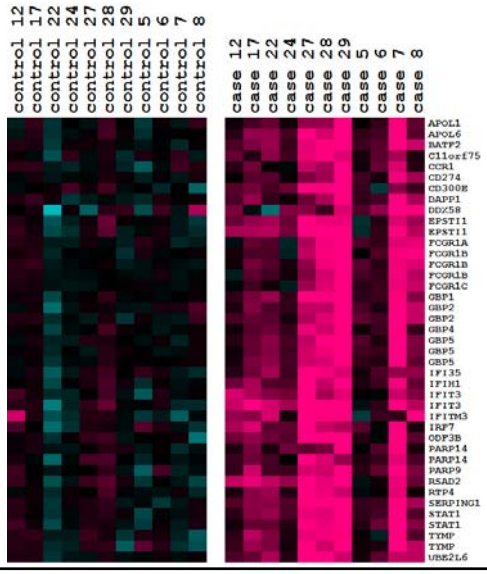

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*RNA-Seq using RNA from Paxgene tubes, 6 months prior to TB Dx.

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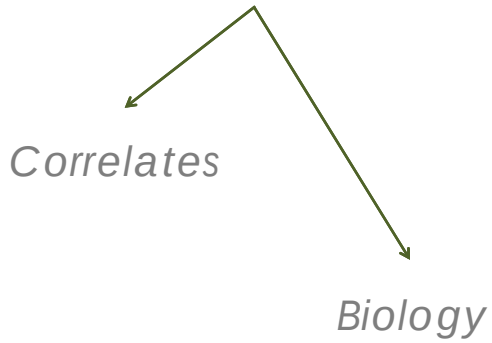
Bioinformatics approaches....3.



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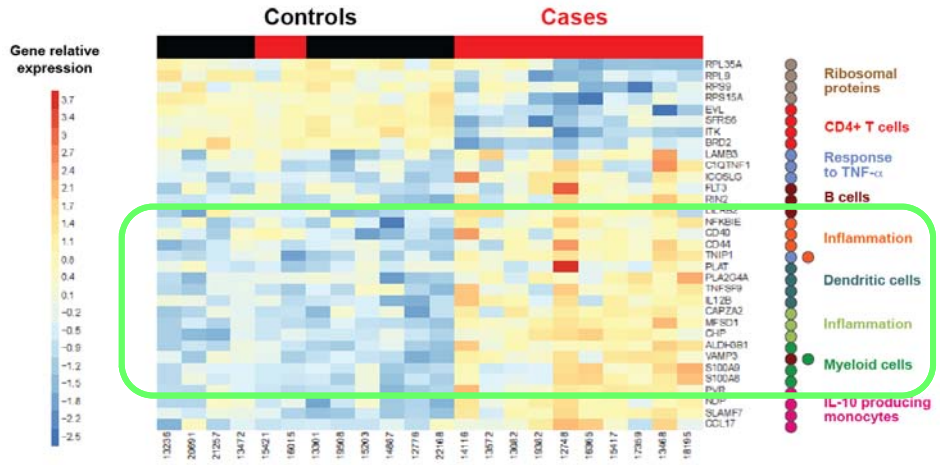
Myeloid cell activation and inflammation associate with risk of TB, after BCG



Helen Fletcher, Ali Filali, Rafick-Pierre Sekaly, many others.
*GSEA pathways from, DNA microarray analysis of RNA from PBMC from 10 wks of age, incubated with BCG for 12 hours.

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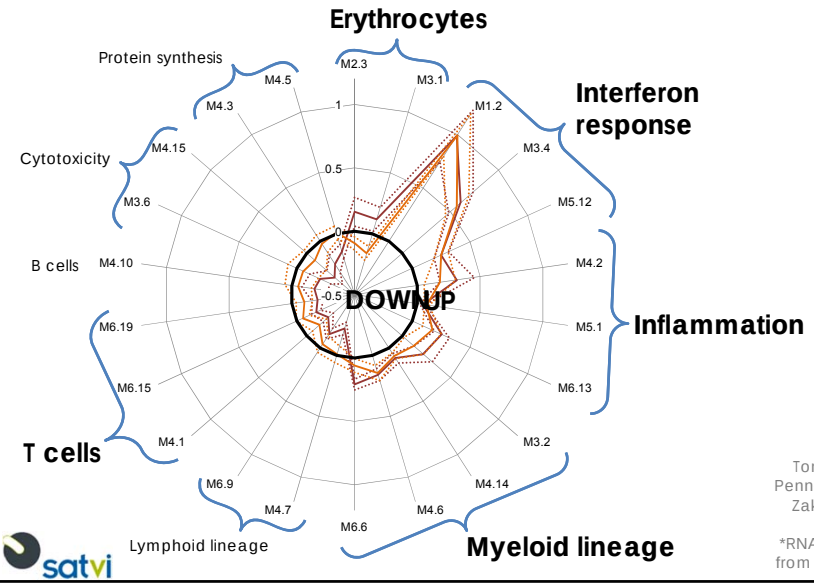
Expression pathways: 6 mo prior to TB Dx in adolescents



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Bioinformatics approaches....4.

Validation is critical!

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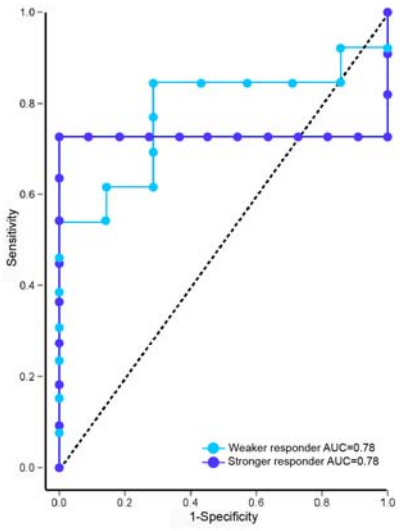
Validated expression signatures that associate with risk of TB, after BCG



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*qPCR analysis of RNA from PBMC from 10 wks of age, incubated with BCG for 12 hours.

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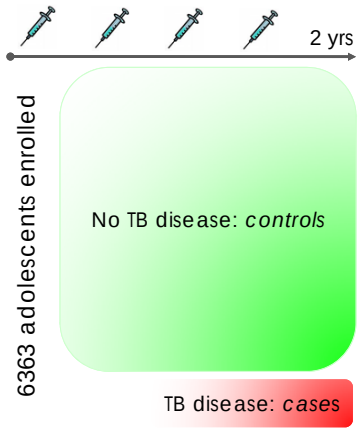
To determine correlates of risk of TB disease, following infection with *Mtb*



TRAINING SET | **VALIDATION SET**

- **Cross-sectional Analysis**

Cases: n = 35
Controls: n = 70

Cases: n = 14
Controls: n = 28

- **Longitudinal Analysis**

Cases: n = 28
Controls: n = 28

Cases: n = 8
Controls: n = 8

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Tom Scriba, Adam Penn-Nicholson, Hassan Mahomed, Dan Zak, Alan Aderem, many others.

Table 1. Terminology for immunological measurements as 3 levels of immune correlates—correlate of risk (CoR), level 1 surrogate of protection (SoP), and level 2 SoP.

Term	Definition	Framework for empirical assessment	Data analytic methods
CoR	An immunological measurement that correlates with the rate or level of a study end point used to measure VE in a defined population	Efficacy trials or observational studies	Regression models
Level 1 SoP	An immunological measurement that is a CoR within a defined population of vaccinees and is predictive of VE in the same setting as the trial; validation entails showing either level 1 SoP^S or level 1 SoP^P		
Level 1 SoP^S	The relationship between the immunological measurement and the risk of the study end point is the same in vaccinees and nonvaccinees	Single large VE trial	Statistical surrogate framework [16]
Level 1 SoP^P	The criterion defined by Frangakis and Rubin [18] and by P.B.G. and M. Hudgens (unpublished data): (1) groups of subjects with no or the lowest vaccine effect on the immune response have no VE and (2) groups of subjects with a sufficiently large vaccine effect on the immune response have positive VE	Single large VE trial	Principal surrogate framework [18–21]
Level 2 SoP	An immunological measurement that is a level 1 SoP and that is predictive of VE in different settings (e.g., across vaccine lots, across human populations, across viral populations, across species)	Multiple VE trials and/or postlicensure studies	Meta-analysis [11–15]

NOTE. SoP^P, SoP principal; SoP^S, SoP statistical; VE, vaccine efficacy.

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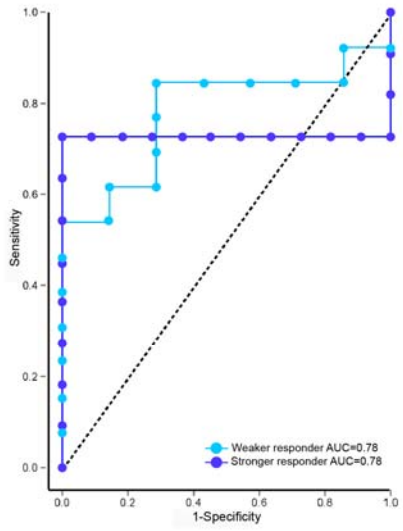
L Qin JID 2007;196:1304

Validated expression signatures that associate with risk of TB, after BCG



Helen Fletcher, Ali Filali, Rafick-Pierre Sekaly, many others.
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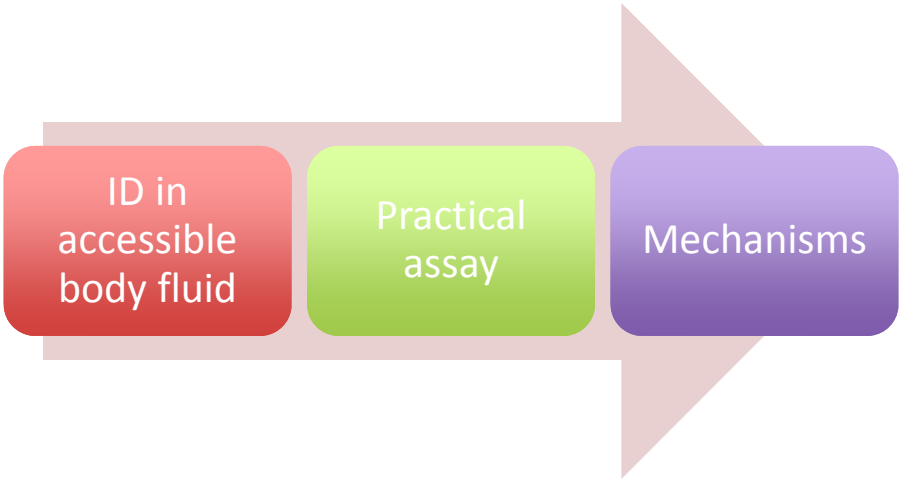
Why do the biomarker study? Common steps in biomarker discovery



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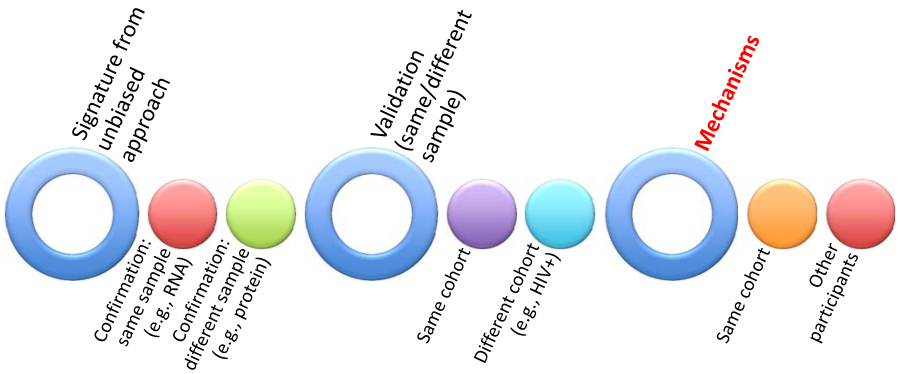
Modified, after M Disis 2011; Cancer Immunol Immunother 66:433.



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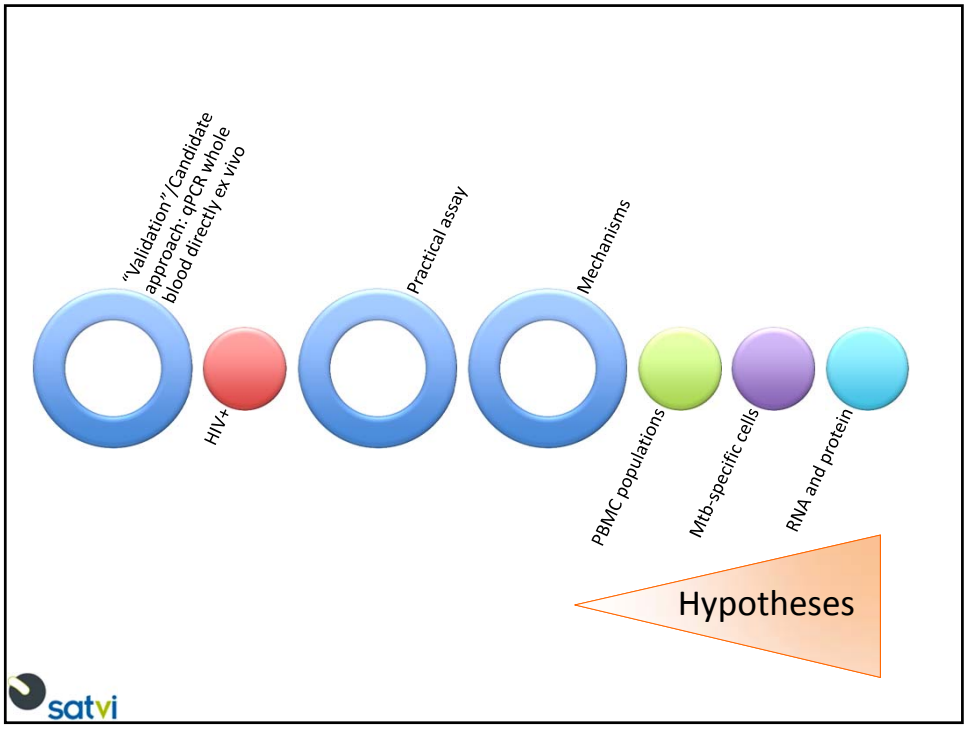
"Validation"/Candidate approach: qPCR whole blood directly ex vivo
HIV+
Practical assay
Mechanisms
PBMC populations
Mtb-specific cells
RNA and protein
Hypotheses
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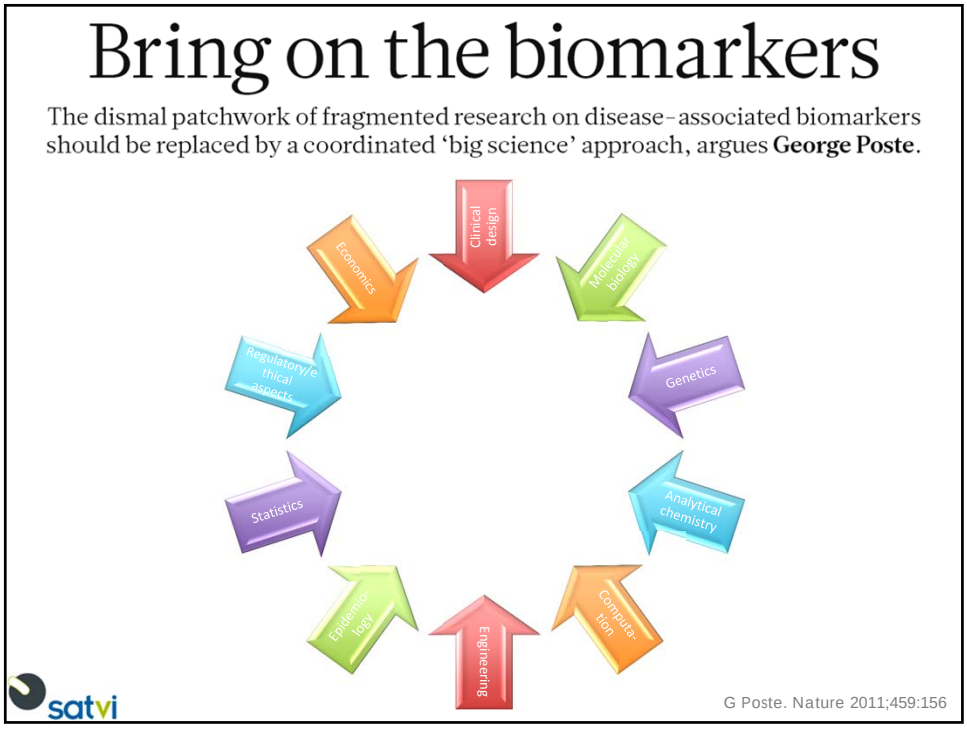
Bring on the biomarkers
The dismal patchwork of fragmented research on disease-associated biomarkers should be replaced by a coordinated 'big science' approach, argues George Poste.
Clinical design
Molecular biology
Genetics
Analytical chemistry
Computation
Engineering
Epidemiology
Statistics
Regulatory/ethical aspects
Economics
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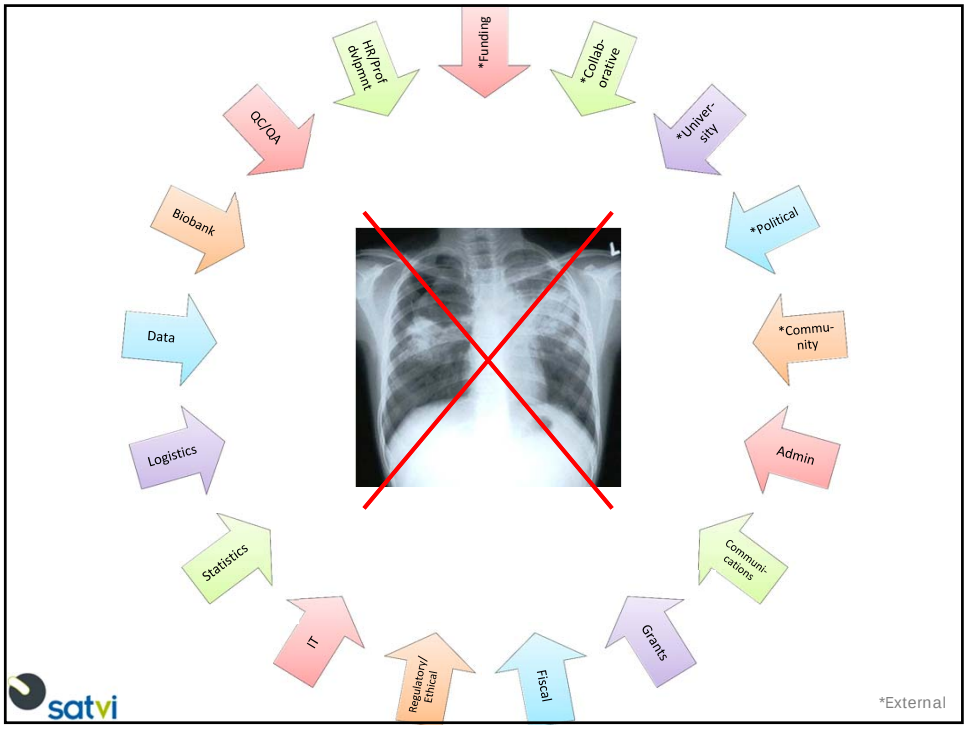
G Poste. Nature 2011;459:156



A few methodological lessons learnt

1. The clinical phenotypes are the most NB!
2. Pay particular attention to sample size
3. Make sure validation cohort adequate
4. Immediate sample processing needs to be optimal
5. Optimal biobanking indispensible
6. Define everything beforehand!
7. Regardless, methods WILL change during the study!

Funders and partners