Host-related markers of response to TB treatment

Dr. Delia Goletti
National Institute for Infectious Diseases L. Spallanzani, Italy
April 14th, 2019
Conflict of interest

In the last year I have been a consultant or I presented talks for:

Diasorin
Janssen
Qiagen
Quidel
Host markers

- Immune cell profiles CD27 expression of T-cells
- CD38/HLA-DR/Ki67 expression of M. tuberculosis-specific T-cells
- IGRA (megapools of peptides; HBHA; ESAT-6; CFP-10)
- Signature
- PET/CT-SCAN
- Urine Metabolite as a Seryl-Leucine Glycopeptide

Microbioma

- Microbioma
TB biomarkers: correlates of response to therapy

b. Correlate of TB disease

c. Correlate of Response to TB Treatment

3-5% of relapses after TB cure
Cured tuberculosis

<table>
<thead>
<tr>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cured TB</td>
</tr>
<tr>
<td>Smear- or culture-negative sputum specimens in the last month of treatment and on at least one previous occasion, according to WHO guidelines.</td>
</tr>
<tr>
<td>Recurrent TB disease</td>
</tr>
<tr>
<td>Refers to a repeat occurrence of TB disease in a patient that occurs as a result of either relapse or re-infection. Recurrent TB occurs after the previous/initial episode has been classified as clinically cured according to WHO guidelines.</td>
</tr>
<tr>
<td>Re-infection</td>
</tr>
<tr>
<td>Recurrent TB disease may occur as a result of re-infection, whereby a patient is exogenously infected with a Mycobacterium tuberculosis strain that is either the same or distinct from the organism that caused the original infection.</td>
</tr>
<tr>
<td>Relapse</td>
</tr>
<tr>
<td>Defined as a second (or third) episode of active TB disease due to re-emergence of the original infection, as determined by genotypic analysis of the prevailing tubercle bacilli.</td>
</tr>
</tbody>
</table>
Can we determine relapse-free cure during treatment?

<table>
<thead>
<tr>
<th>Test</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiology</td>
<td></td>
</tr>
<tr>
<td>AFB conversion from positive to negative</td>
<td>S</td>
</tr>
<tr>
<td>Negative cultures after 2 and 6 months during TB therapy</td>
<td>S</td>
</tr>
<tr>
<td>Early bactericidal activity (BACTEC-MGIT 960)</td>
<td>S</td>
</tr>
<tr>
<td>DNA detection (PCR; GenXpert MTB/RIF test)</td>
<td>R</td>
</tr>
<tr>
<td>RNA detection (isocitrate lyase mRNA; M. tuberculosis rRNA; sets of mRNA signatures)</td>
<td>R</td>
</tr>
<tr>
<td>Immunology</td>
<td></td>
</tr>
<tr>
<td>Monocyte/lymphocyte ratio</td>
<td>R</td>
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<tr>
<td>CD27 expression of T-cells</td>
<td>R</td>
</tr>
<tr>
<td>CD38/HLA-DR/Ki67 expression of M. tuberculosis-specific T-cells</td>
<td>R</td>
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<tr>
<td>M-MDSC</td>
<td>R</td>
</tr>
<tr>
<td>Levels of inflammatory molecules (IP-10; CRP; β2-microglobulin; a seven-molecule signature)</td>
<td>R</td>
</tr>
<tr>
<td>IGRA (megapools of peptides; HBHA; ESAT-6; CFP-10)</td>
<td>R</td>
</tr>
<tr>
<td>Radiology</td>
<td></td>
</tr>
<tr>
<td>CT scan</td>
<td>S</td>
</tr>
<tr>
<td>PET/CT scan</td>
<td>R</td>
</tr>
</tbody>
</table>

CT: computed tomography; PET: positron emission tomography; AFB: acid-fast bacilli; S: standard; R: research; M-MDSC: monocytic myeloid-derived suppressor cells; IP: interferon-γ induced protein; CRP: C-reactive protein; IGRA: interferon-γ release assay; HBHA: heparin-binding haemagglutinin; ESAT: early-secreted antigenic target; CFP: culture filtrate protein.
Adaptive immunity in TB: classical and non-classical T cells

From Tom Ottehoff, personal
There is always a balance....

Increased inflammation
Tissue damage
*M. tuberculosis* load increased

Inflammation controlled
*M. tuberculosis* load contained
## T cell maturation

<table>
<thead>
<tr>
<th>Immune state</th>
<th>CD45RA</th>
<th>CD45RO</th>
<th>CCR7</th>
<th>CD62L</th>
<th>CD28</th>
<th>CD27</th>
<th>IL-7Rx</th>
<th>CXCR3</th>
<th>CD95</th>
<th>CD11a</th>
<th>IL-2Rβ</th>
<th>CD58</th>
<th>CD57</th>
</tr>
</thead>
<tbody>
<tr>
<td>TN</td>
<td>+</td>
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<tr>
<td>TSCM</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>TCM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>TEM</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>TE</td>
<td>+</td>
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</tbody>
</table>

### Key Features
- Stemness
- Proliferative potential
- Lymphoid homing
- Antigen independence
- Lipid metabolism
- Low Δψm
- Senescence
- Cytotoxicity
- Tissue tropism
- Antigen addiction
- Glycolytic metabolism
- Oxidative stress
CD27 modulation within the CD45RA- cells helps to discriminate among the different TB stages

Petruccioli et al., J Infection, 2015
CD27 modulation: a potential new biomarker for TB?

\[
\text{RATIO MFI} = \frac{\text{MFI}_{\text{CD27 gate of CD4}^+ \text{ T cells}}}{\text{MFI}_{\text{CD27 gate of CD4}^+ \text{IFN}^+ \text{ T cells}}}
\]
Bifunctional IFN-γ and TNF-α CD4 cells responding to RD1 proteins and an effector memory phenotype associate with active TB

**HIV-uninfected**

- Active TB
- Cured TB
- LTBI

**Cytokine Response**

- IFNγ
- IL2
- TNFα

**Phenotype**

- EM
- CM

**HIV-infected**

- Active TB
- LTBI

Petruccioli and Petrone et al, J Infection 2013
Chiacchio and Petruccioli et al, J Infection 2014
Combination of tests increases diagnostic accuracy

Petruccioli et al, Diagn Microbiol Infect Dis., 2016
The proportion of HLA-DR\(^+\) M. tuberculosis-specific CD3+ T-cells co-expressing IFN\(\gamma\) and TNF\(\alpha\) associates to active TB

Musvosvi et al, ERJ, 2018
Longitudinal monitoring of the frequencies of CD38 in Mtb-specific CD4+ T cells is a useful biomarker for TB cure

CD8⁺ T-cell frequency decreases in active TB patients after TB-specific therapy

Day et al, J Immunol 2011
# QuantiFERON-TB Gold vs QuantiFERON-TB Gold PLUS

<table>
<thead>
<tr>
<th>Blood collection</th>
<th>QuantiFERON® TB Gold In tube</th>
<th>QuantiFERON® TB Gold Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td>nil</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>TB antigen</td>
<td>TB antigen</td>
<td>TB 1</td>
</tr>
<tr>
<td></td>
<td>CD4⁺ T-cells</td>
<td>CD4⁺ and CD8⁺ T-cells</td>
</tr>
<tr>
<td>mitogen</td>
<td>mitogen</td>
<td>mitogen</td>
</tr>
</tbody>
</table>

## Peptides

<table>
<thead>
<tr>
<th>Type</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESAT-6</td>
<td>polypeptides</td>
</tr>
<tr>
<td>CFP-10</td>
<td>polypeptides</td>
</tr>
<tr>
<td>TB7.7</td>
<td>polypeptides</td>
</tr>
</tbody>
</table>

- ESAT-6 polypeptides
- CFP-10 polypeptides
- TB7.7 polypeptides

+ additional short peptides
QFT-Plus: TB 1. CD4 response in all groups (active TB and LTBI).
QFT-Plus: TB 2. CD4 response in all groups and CD8 response in active TB.

<table>
<thead>
<tr>
<th></th>
<th>CD4</th>
<th></th>
<th>CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB1 N (%)</td>
<td>TB2 N (%)</td>
<td>(N)</td>
<td>TB1 N (%)</td>
</tr>
<tr>
<td>19 (83)</td>
<td>21 (91)</td>
<td>ACTIVE TB (23)</td>
<td>4 (17)</td>
</tr>
<tr>
<td>17 (94)</td>
<td>15 (83)</td>
<td>LTBI REMOTE (18)</td>
<td>3 (17)</td>
</tr>
<tr>
<td>12 (100)</td>
<td>11 (92)</td>
<td>LTBI RECENT (12)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>48 (90.5)</td>
<td>47 (89)</td>
<td>TOTAL (53)</td>
<td>11 (21)</td>
</tr>
</tbody>
</table>
IFNγ response to TB1 and TB2 of the QFT-Plus decreases after therapy in patients with active TB

TABLE 1 Interferon-γ test quantitative and qualitative results

<table>
<thead>
<tr>
<th></th>
<th>0 months</th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quantitative data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB1 (surrogate CD4* T-cell response) IU·mL⁻¹</td>
<td>6.40±8.92</td>
<td>2.56±3.28*</td>
<td>2.33±3.06</td>
</tr>
<tr>
<td>TB2 (CD4 and CD8 response) IU·mL⁻¹</td>
<td>8.98±16.25</td>
<td>4.50±7.53*</td>
<td>3.23±4.95</td>
</tr>
<tr>
<td>TB2-TB1 (surrogate CD8* T-cell response) IU·mL⁻¹</td>
<td>2.58±8.45</td>
<td>1.93±5.12</td>
<td>0.91±2.85*</td>
</tr>
<tr>
<td><strong>Qualitative data</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB1 positive/negative/indeterminate</td>
<td>35/2/1</td>
<td>31/7/0</td>
<td>26/11/1</td>
</tr>
<tr>
<td>TB2 positive/negative/indeterminate</td>
<td>36/1/1</td>
<td>32/6/0</td>
<td>32/5/1</td>
</tr>
<tr>
<td>TB1 or TB2 positive/negative/indeterminate</td>
<td>36/1/1</td>
<td>32/6/0</td>
<td>32/5/1</td>
</tr>
</tbody>
</table>

Quantitative data are presented as mean±so. TB1: QFT-Plus Tube 1; TB2: QFT-Plus Tube 2. *: p<0.05 compared to prior value.
IFNγ response to TB1 and TB2 of the QFT-Plus decreases after therapy in patients with active TB

Petruccioli et al, Scientific Report, 2018
In patients with active TB the response to RD1 selected peptides decreases after efficacious treatment.

M. Amicosante

Carrara et al, CID 2004
Response to RD1 selected peptides decreases after efficacious treatment in TB-HIV-infected patients in Uganda

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity for active HIV-TB %</th>
<th>Specificity for active HIV-TB %</th>
<th>OR for active HIV-TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>RD1 selected peptides</td>
<td>69</td>
<td>93</td>
<td>29</td>
</tr>
<tr>
<td>TST</td>
<td>46</td>
<td>61</td>
<td>1</td>
</tr>
<tr>
<td>T SPOT TB</td>
<td>84</td>
<td>64</td>
<td>10</td>
</tr>
<tr>
<td>QFT-G</td>
<td>84</td>
<td>75</td>
<td>17</td>
</tr>
</tbody>
</table>

Vincenti et al, CEI, 2007

Goletti et al, BMC ID 2008
Response to RD1 selected peptides decreases after efficacious treatment in TB patients in India

RD1 selected peptides

IP-10 vs IFN-γ

Kabeer et al, BMC ID 2011

ESCMID eLibrary © by author
Modulation of HBHA response is associated with TB development or control
IFN-γ response to QFT antigens and HBHA in response in children with LTBI and active TB

45 LTBI
19 active TB

Sali M, J. Infection, 2018
Following TB-specific therapy, most of the non-HBHA-responding children, gained an HBHA-positive response.

HBHA-based IGRA To Monitor TB Therapy
IFN-γ response to HBHA with active TB before and after successful therapy

Drug-susceptible TB cases (n=17)

Preliminary data from Bangladesh

Wen et al, Eur J Clin Microb Inf Dis, 2017
Mycobacterial growth inhibition assays (MGIA) surrogate of protective immunity to TB

MGIA$s utilise whole blood or peripheral blood mononuclear cells (PBMC$s), and measure the ability to inhibit growth of mycobacteria following in vitro infection
Mycobacterial growth inhibition assay result (MGIA) associates with TB stages
Mycobacterial growth inhibition assay result (MGIA) is modulated by therapy in active TB patients

O’Shea et al, Scientific Report, 2018
The blood transcriptional signature changes during different phases of treatment

Cliff at al, Immunological Reviews 2015
Complement Component C1q as Serum Biomarker to Detect Active Tuberculosis

Lubbers et al, Front Immunol, 2018
Complement Component C1q as Serum Biomarker to Detect Active Tuberculosis
A blood RNA signature for tuberculosis disease risk: a prospective cohort study

Zak at al, Lancet, 2016
Symmetry between progression and treatment response signatures

Progression signature

Disease signature

Walzl G et al, TBScience, 2018
A subset of treated patients remains with incipient/subclinical TB

*Risking TB burden implies an increase in abundance of TB and pathogen biomarkers, compartment-specific changes in immunological responses, and a decrease in the probability of disease resolution in the absence of treatment.
Which implies that...

- Biomarker signatures that are useful for triaging and TB prediction may also be useful for predicting relapse-free cure.

- Assays based on these signatures may require different performance thresholds for different applications.

- Combining pretreatment stratification with testing during treatment may allow a substantial proportion of TB patients to be treated for only 2 months.

- We need to do trials of host-stratification strategies.
Host markers

- Immune cell profiles CD27 expression of T-cells
- CD38/HLA-DR/Ki67 expression of M. tuberculosis-specific T-cells
- IGRA (megapools of peptides; HBHA; ESAT-6; CFP-10)
- Signature
- PET/CT-SCAN
- Urine Metabolite as a Seryl-Leucine Glycopeptide

Microbioma
Persisting positron emission tomography lesion activity and *M. tuberculosis* mRNA after tuberculosis cure

- A subset of clinically and microbiologically cured patients 6M of treatment and 12 months after treatment completion
- Evidence for non-sterilizing cure after clinically curative treatment
  - Imaging: ongoing inflammation
  - *M.tb* mRNA presence in respiratory secretions
  - Differentially culturable bacteria
- Viable non-culturable bacilli presence in line with relapse rates.

*Malherbe et al, Nature Medicine, 2016*
Human Urine Metabolite as a Seryl-Leucine Glycopeptide and as a Biomarker of Effective Anti-Tuberculosis Therapy

Liquid chromatography−mass spectrometry

* Liquid chromatography−mass spectrometry

Fitzgerald et al, ACS Infectious Diseases, 2019
SLC1G levels are associated with active TB and different treatment response outcomes
SLC1G levels associate with clinical measurements of bacterial burden and inflammation.

Fitzgerald et al, ACS Infectious Diseases, 2019
<table>
<thead>
<tr>
<th>Host markers</th>
<th>Microbioma</th>
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<tbody>
<tr>
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<td>PET/CT-SCAN</td>
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<tr>
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<td></td>
</tr>
</tbody>
</table>
The microbiome and tuberculosis

The diverse microbial communities within our bodies produce metabolites that modulate host immune responses

Naidoo et al, Lancet ID, 2019
First-line TB treatment appears to have little overall effect on bacterial diversity in the gut, although the relative abundances of specific taxa are altered. Many of these taxa are previously documented host immune modulators:

- **Bacteroides**-produced polysaccharides mediate mucosal tolerance via upregulation of Tregs,
- **Lactobacillus** spp modulate innate and adaptive immune responses via direct binding to pattern recognition receptors,
- **Prevotella** are linked to enhanced Th17-cell mediated inflammation.
The microbiome and tuberculosis

Naidoo et al, Lancet ID, 2019
Microbiota and TB clinical outcome

Drug absorption and metabolism
• How is the microbiome affected by drug metabolism?
• Can the microbiome itself affect the metabolism of specific drugs, thereby altering treatment outcomes?

Clinical outcome
• Are specific microbial signatures indicative of a favourable or unfavourable treatment outcome?
• By what mechanism do microbial signatures predict treatment outcome?
• Does the microbiome influence risk of tuberculosis recurrence?
From Anthony Fauci: we need to “reimagine” our research response to TB and bring TB research into the 21\textsuperscript{st} century (Moscow, 2017)

AS Fauci outlined how we might “reimagine” our research response to TB and bring TB research into the twenty first century with the application of new diagnostic, therapeutic, and vaccine platforms. The current situation with TB research contrasts dramatically with the unprecedented advances in HIV/AIDS research made in the > 36 years since HIV was first reported.

<table>
<thead>
<tr>
<th></th>
<th>HIV</th>
<th>TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>HIV-kit available, rapid and accurate, in low income countries included</td>
<td>Need to be improved</td>
</tr>
<tr>
<td>Therapy</td>
<td>30 drugs approved</td>
<td>Less than 10</td>
</tr>
<tr>
<td>Biomarkers for TB treatment monitoring, cure and relapse</td>
<td>Available</td>
<td>Partly available</td>
</tr>
</tbody>
</table>

Anthony S. Fauci and Robert W. Eisinger, Am J Trop Med, 2018
National Institute for Infectious Diseases (INMI) L. Spallanzani, Rome, Italy

HIV: 6,800-7,000 (300 new infection)
HCV: 1,500-2,000
HBV: 800-1,000
Active TB: 300-350, LTBI: 200; HIV-TB: 40
Thank you

Translational Research Unit
Teresa Chiacchio
Gilda Cuzzi
Linda Petrone
Elisa Petruccioli
Valentina Vanini
Tonino Alonzi

INMI
Enrico Girardi
Fabrizio Palmieri
Andrea Antinori
Vincenzo Schininà
Giuseppe Ippolito