Predictive biosignatures to improve tuberculosis vaccine development

Stefan H.E. Kaufmann
Max Planck Institute for Infection Biology, Berlin, Germany

5th Global Forum on TB Vaccines

Session: The Cutting Edge: Translating Scientific Advances into New TB Vaccines

20 - 22 February 2018, New Delhi
Kaufmann et al., 2017
Let’s start with transcripts - the most widely used biomarkers in TB...

...and the role of computational modelling.

Kaufmann et al., 2017
AUC (95% CI):

TB vs other:
AUC=0.95 (0.93-0.96)

TB vs healthy:
AUC=0.92 (0.90-0.94)
TB Biomarkers

Where are we?

- Small signatures comprising 3-4 transcripts (decision trees or pairwise clustering) can reliably diagnose active TB from LTBI.
  - Maertzdorf et al., 2016; Sweeney et al., 2016
- Signatures comprising ~16 transcripts can predict active TB in individuals with LTBI 6-12 months prior to clinical diagnosis.
  - Zak et al., 2016; Suliman et al., submitted

From small numbers to big data…

Or: from outliers to biological relevance
TB Biomarkers

Where are we?

- Small signatures comprising 3-4 transcripts (decision trees or pair wise clustering) can reliably diagnose active TB from LTBI.

Maertzdorf et al., 2016; Sweeney et al., 2016

- Signatures comprising ~16 transcripts can predict active TB in individuals with LTBI 6-12 months prior to clinical diagnosis.

Zak et al., 2016; Suliman et al., submitted

Can we harness gene expression profiles for identifying an appropriate animal model for TB vaccines?

A proof of concept study....
Battle of the mouse model

- Seok et al., PNAS (2013)
- all orthologous gene pairs included in analysis
- correlation coefficient of determination ($r^2$)
<table>
<thead>
<tr>
<th>Genomic responses in mouse models poorly mimic human inflammatory diseases</th>
<th>Genomic responses in mouse models greatly mimic human inflammatory diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Seok et al., PNAS (2013)</td>
<td>- Takao et al., PNAS (2015)</td>
</tr>
<tr>
<td>- all orthologous gene pairs included in analysis</td>
<td>- mouse models mimic partial aspects of human disease</td>
</tr>
<tr>
<td>- correlation coefficient of determination ($r^2$)</td>
<td>- Spearman’s rank correlation coefficient ($r$)</td>
</tr>
</tbody>
</table>
Differential Susceptibility to TB of C57BL/6 and 129S2 mice

Survival post low dose aerosol infection (schematic)

Days post infection
Survival (%)
0 20 40 60 80 100

C57BL/6
129S2

Dorhoi, Yeremeev et al., EJI, 2014
Algorithm for identifying concordant and discordant gene sets

Domaszewska et al., Scientific Reports 2017
How similar is gene regulation?

\[
disco.\, score = \log_2 FC_{Hs} \cdot \log_2 FC_{Mm} \cdot |(\log_{10} P_{Hs} + \log_{10} P_{Mm})|
\]

TB patients and 129S2 mice (whole blood)

With time after infection of mice, 129S2 mice present more concordances and less discordances with TB patients.

<table>
<thead>
<tr>
<th></th>
<th>No. Concordant Genes</th>
<th>No. Discordant Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The Gambia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>day 7</td>
<td>53</td>
<td>8</td>
</tr>
<tr>
<td>day 14</td>
<td>66</td>
<td>28</td>
</tr>
<tr>
<td>day 21</td>
<td>106</td>
<td>5</td>
</tr>
<tr>
<td><strong>South Africa</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1</td>
<td>36</td>
<td>28</td>
</tr>
<tr>
<td>day 7</td>
<td>66</td>
<td>14</td>
</tr>
<tr>
<td>day 14</td>
<td>67</td>
<td>24</td>
</tr>
<tr>
<td>day 21</td>
<td>104</td>
<td>4</td>
</tr>
<tr>
<td><strong>Malawi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1</td>
<td>28</td>
<td>36</td>
</tr>
<tr>
<td>day 7</td>
<td>49</td>
<td>16</td>
</tr>
<tr>
<td>day 14</td>
<td>60</td>
<td>25</td>
</tr>
<tr>
<td>day 21</td>
<td>102</td>
<td>2</td>
</tr>
<tr>
<td><strong>C57BL/6</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>day 7</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>day 14</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>day 21</td>
<td>85</td>
<td>24</td>
</tr>
</tbody>
</table>

The Gambia: Maertzdorf et al
South Africa: Maertzdorf et al
Malawi: Kaforou et al

Domaszewska et al., Scientific Reports 2017
TB patients and C57BL/6 mice (whole blood)

Discordances between C57BL/6 mice and TB patients are larger than for 129S2 mice and decrease less with time after infection.

Concordances at every time point after infection are smaller than between 129S2 mice and TB patients.
Among concordant genes between 129S2 mice and man, which are discordant in C57BL/6 mice, T-cell co-receptor genes are overrepresented

- **CD28**: Costimulatory receptor on naïve T cells for B7 (CD80 and CD86) on APC
- **CD3d**: δ chain of the CD3 molecule associated with TCR
- **CD3ε**: ε chain of the CD3 molecule associated with TCR
- **CD3γ**: γ chain of the CD3 molecule associated with TCR
- **CD5**: Expressed on T cells and upregulated upon strong activation
- **CD7**: Member of immunoglobulin superfamily on mature T cells involved in T cell activation
- **CD96**: Member of the immunoglobulin superfamily on T cells involved in adhesion of activated T cells to target cells at sites of inflammation

Domaszewska et al., Scientific Reports 2017
Conclusions

• **Differential** gene regulation in response to M.tb in C57BL/6 and 129S2 mouse strains.

• AT 21 days p.i the response in blood cells of 129S2 mice concordant, of C57BL/6 mice discordant, to that of TB patients.

• **Discordant** gene regulation in response to M.tb in TB patients and C57BL/6 mouse mostly related to T-cell functions.

• **Innate immune functions** concordant between human and murine species, in both 129S2 and C57BL/6 mice.
Conclusions

• **Differential** gene regulation in response to M.tb in C57BL/6 and 129S2 mouse strains.

• AT 21 days p.i the response in blood cells of 129S2 mice concordant, of C57BL/6 mice discordant, to that of TB patients.

• Discordant gene regulation in response to M.tb in TB patients and C57BL/6 mouse mostly related to T-cell functions.

• **Innate** immune functions concordant between human and murine species, in both 129S2 and C57BL/6 mice.

Based on concordant and discordant transcripts we could select an animal model that mimics human TB.

Similar strategy for selection of TB vaccine model.
Conclusions

• **Differential** gene regulation in response to M.tuberculosis (M.tb) in C57BL/6 and 129S2 mouse strains.

Let's now move to prediction of human TB....

Samples from two studies, also used for cross validation:

• Adolescent cohort study (ACS)
• Multicentric household contact study (GC-6)

• Innate immune functions concordant between human and murine species, in both 129S2 and C57BL/6 mice.

Domaszewska et al., Scientific Reports 2017
## Strategy towards a prognostic biosignature for risk of TB

D. Zak et al., Lancet, 2016

<table>
<thead>
<tr>
<th>Adolescent cohort for training and test set</th>
<th>Grand Challenge 6 cohort for validation set</th>
</tr>
</thead>
<tbody>
<tr>
<td>6,363 participants</td>
<td>4,466 participants</td>
</tr>
<tr>
<td>46 TB progressors</td>
<td>73 TB progressors</td>
</tr>
<tr>
<td>37 TB progressors in training group</td>
<td>43 TB progressors from South Africa</td>
</tr>
<tr>
<td>9 TB progressors in test group</td>
<td>30 TB progressors from The Gambia</td>
</tr>
</tbody>
</table>
Transcript signature from adolescent cohort study (training set) predicts risk of TB in GC6 study (validation set)

Zak et al, Lancet, 2016
Transcript signature from adolescent cohort study (training set) predicts risk of TB in GC6 study (validation set)

Zak et al, Lancet, 2016
Transcript signature from adolescent cohort study (training set) predicts risk of TB in GC6 study (validation set)

Let’s now move to prediction ....

Samples from two studies, used for cross validation:

- Adolescent cohort study (ACS)
- Multicentric household contact study (GC-6)

Zak et al, Lancet, 2016
Strategy towards a prognostic biosignature for risk of TB

Grand Challenge 6 cohort study for validation

4,466 participants

100 TB progressors

43 TB progressors from South Africa (3.6%)

34 TB progressors from The Gambia (1.7%)

12 TB progressors from Ethiopia (1.5%)

11 TB progressors from Uganda (2.2%)

Suliman et al., submitted
Prediction of Active TB Progression by Transcriptomic GC6 Biosignatures

Suliman et al., submitted
Prediction of Active TB Progression by Transcriptomic GC6 Biosignatures

Suliman et al., submitted
Prediction of Active TB Progression by Transcriptomic GC6 Biosignatures

Biosignature for TB risk
Can we further minimize the risk signature?

Yes, by introducing pair ratios (upregulated marker/dowregulated marker)

Suliman et al., submitted
Prediction of TB Progression by Pair Ratios Alone or Together

Suliman et al., submitted
Prediction of TB Progression by Pair Ratios Alone or Together

Suliman et al., submitted
Prediction of TB Progression by Pair Ratios Alone or Together

These were transcripts. What about metabolites?

Suliman et al., submitted
Prediction of TB Progression by a Metabolite-Based GC6 Biosignature vs. Healthy Individuals

Weiner et al., submitted
The principal component which corresponds to differences between cases and controls shows divergence emerging at around 12 to 11 months prior to the clinical diagnosis.

Weiner et al, in preparation

Weiner et al., submitted
Predictive TB Biomarkers

- Metabolomic and transcriptomic signatures can be harnessed for both diagnosis and prognosis of TB.
- Prognostic biomarkers arise over time: in reality detect disease progression (6-12 months prior to active disease)
- Original signature: 16 transcripts
- Decision trees & pair ratios: down to 1 to 2 marker pairs
- Further optimization: Multiplatform analysis

Zak et al., Lancet, 2016; Suliman et al., submitted; Weiner et al., submitted; Duffy et al., submitted
Day 0
- IGRA, TST
- Omics

Years
- Omics

Months
- Omics

Day X
- Clinical diagnosis
  X-Ray, Omics

Containment of dormant Mtb in solid granulomas

Containment of dormant Mtb Transition to necrosis/caseation

Caseous granuloma with active Mtb
But: We are ambitious…

- Individuals with subclinical TB deserve offer for preventive therapy.
- Individuals with subclinical TB may have reached a point of no return.
- Thus, the bar is high.
- Alternative: exclude subclinical TB. We need to balance caution and risk.
TB Biomarkers

What is needed now?

• Signatures of *vaccine efficacy*.

• Signatures of *vaccine safety*. 

[Image of vaccination scene]
BioVacSafe

**Biomarkers for Vaccine immunoSafety**

Innovative Medicines Initiative

**Call topic:** Immunosafety Of Vaccines – New Biomarkers Associated With Adverse Events (Early Inflammation, Autoimmune Diseases And Allergy)

**Budget:** 30.4M€

**Duration:** 5+1 years March 2012 – 2018

www.biovacsafe.eu
TB Biomarkers

What is needed now?

• Signatures of vaccine efficacy.

• Signatures of vaccine safety.
What we need for TB vaccine signatures:

- Collection and computational analysis of samples from ongoing vaccine trials.
- Biorepositories for future state-of-the-art studies/analyses.
- Correlation with clinical outcome (healthy vs. diseased vaccinees and controls).
Collaborators:

- John Kenneth, Bangalore
- Erik Lader, Qiagen
- Gerhard Walzl, SUN
- Willem Hanekom, SATVI
- Thomas Scriba, SATVI
- Sara Suliman, SATVI
- Daniel Zak, CIDR
- Fergal Duffy, CIDR
- Ethan Thompson, CIDR
- Jayne Sutherland, MRC
- Hazell Dockrell, LSHTM

Jeroen Maertzdorf, MPIIB
January Weiner, MPIIB

Grand Challenges in Global Health
Biomarkers of Protective Immunity against TB in the context of HIV/AIDS in Africa (GC6-74)

UCI
Willem Hanekom
Tom Scriba
Hassan Mahomed
Jane Hughes

Stanford Univ.
Gary Schoolnik
Gregory Dolganov
Tran Van

EHNRI
Desta Kassa
Almaz Abebe
Tsehayenesh Mesele
Belete Tegbaru

UMCU
Debbie van Baarle
Frank Miedema

SSI
Peter Anderson
Ida Rosenkrands
Mark Doherty
Karim Weldingh

AHRI
Rawleigh Howe
Adane Mihret
Abraham Aseffa
Yonas Bekele
Rachel Iwnetu
Mesfin Tafesse
Lawrence Yamuah

MRC Gambia
Martin Ota
Jayne Sutherland
Simon Donkor
Ifedayo Adetifa
Martin Antonio
Toyin Togun
Philip Hill
Richard Adegbola
Tumani Corrah

AERAS
Jerry Sadoff
Donata Sizemore
S Ramachandran
Lew Barker
Mike Brennan
Frank Weichold
Stefanie Muller
Larry Geiter

MPIIB
Stefan H. E. Kaufmann (PI)
Shreemanta Parida
Robert Golinski
Jeroen Maertzdorf
January Weiner
Marc Jacobson

LUMC
Tom Ottenhoff
Michel Klein
Marielle Haks
Kees Franken
Annemieke Geluk
Krista Meijgaarden
Simone Joosten

LSHTM
Hazel Dockrell
Maeve Lalor
Steve Smith
Patricia Gorak-Stolinska
Yun-Gyoung Hur
Ji-Sook Lee

CWRU
W. Henry Boom
Bonnie Thiel
Makerere
Harriet Mayanja-Kizza
Moses Joloba
Sarah Zalwango
Mary Nsereko
Brenda Okware

KPS
Mia Crampin
Neil French
Bagrey Ngwira
Anne Ben Smith
Kate Watkins
Lyn Ambrose
Felanji Simukonda

SUN
Gerhard Walzl
Gillian Black
Gian van der Spuy
Kim Stanley
Daleen Kriel
Nelita Du Plessis
Nonhlanhla Nene Andre
Lxton
Novel Chegou

MRC (Coordinator)
Berlin, Germany

Grand Challenges
in Global Health