Systems immunomonitoring to support development of new treatments and vaccines for TB

Darragh Duffy
Translational Immunology Unit,
Milieu Interieur Consortium,
Institut Pasteur, Paris
C2i, Hong Kong

@darragh_duffy
Disclosures

Received grant support from
Rules Based Medicine
Roche Genentech
Sanofi Pasteur
Understanding immune variability for new clinical strategies

**How are immune responses different in disease?**

- Infection
- Autoimmunity

**How do immune responses differ with age, sex, genetics & environment?**

**What are the cellular mechanisms of these differences?**

**Diagnosis**

**Treatment**

**Vaccination**
Improved reproducibility of whole blood over PBMCs for immunomonitoring

PBMCs removes neutrophils/platelets

PBMCs introduces more technical variability

CyTOF

LPS stimulation

Brodin, Duffy, Quintana Immunity 2019
Functional immune assays to study immune variability

1,000 Healthy donors (Milieu Intérieur)
Piasecka*, Duffy* PNAS, 2018
Duffy, Clin Imm 2017
Urrutia, Duffy Cell Reports 2016
Duffy D, Immunity, 2014

Supernatant
Protein Secretion
Luminex xMAP
Digital ELISA

Cell pellet
Metabolites
Oxylipins by Mass Spectometry

Gene Expression
High-throughput Nanostring RNA-Seq

Cells
Cellular Phenotype
Flow cytometry

Microbes
Cytokines
MAMPs
T cell agonists
Antigens

Patient cohorts (TB, T1D)
Duffy Clin Inf Dis 2021
Rodrigues Diabetologia 2018
TruCulture reveals significant differences between latent infection and active TB disease

TB Diagnosis

TB-Ag - IFNg measured by ELISA

Pre-treatment

Post-treatment – Active TB

Significant difference between LTBI and active TB groups with TruCulture in contrast to QFT

Duffy Clin Inf Dis 2021
TruCulture TB Ag IFNg responses can be used to diagnose active TB

TB Diagnosis

TB-Ag - IFNg measured by ELISA

TruC AUC = 0.81
(95% CI: 0.69-0.93)

QFT AUC = 0.56
(95% CI: 0.40-0.72)

Initial Cohort

Validation Cohort

Truc: AUC = 0.89
(95% CI: 0.82-0.97)

TB Diagnosis

TruCulture TB Ag IFNg responses can be used to diagnose active TB

TB-Ag - IFNg measured by ELISA

TruC AUC = 0.81
(95% CI: 0.69-0.93)

QFT AUC = 0.56
(95% CI: 0.40-0.72)

Initial Cohort

Validation Cohort

Truc: AUC = 0.89
(95% CI: 0.82-0.97)

TruCulture TB Ag IFNg responses can be used to diagnose active TB

TB-Ag - IFNg measured by ELISA

TruC AUC = 0.81
(95% CI: 0.69-0.93)

QFT AUC = 0.56
(95% CI: 0.40-0.72)

Initial Cohort

Validation Cohort

Truc: AUC = 0.89
(95% CI: 0.82-0.97)
Where is the potential clinical value of TruCulture in TB?

**TB Diagnosis**

TB-Ag response > 3 Gene signature (Sweeney et al., 2016) RISK11 (Darboe et al., 2018)

GBP5/DUSP3/KLF2 genes more differential after TB Ag stimulation

Sweeney 3 gene signature

![Graphs showing gene expression changes](image)
Where is the potential clinical value of TruCulture in TB?

TB Diagnosis

TruCulture – ELISA

TruCulture – Digital ELISA

TruCulture – Nanostring

TB-Ag - IFNγ measured by Digital ELISA

4 IFNG^{hi} LTBI donors cluster with active TB disease
Application to TB Predict consortium to identify early response biomarkers

Hypothesis Testing: Can early successful responders to TB treatment be identified by their whole blood induced immune response

TB Predict consortium: Gerhard Walzl, Tom Scriba, Clifton Barry, + many others

Nested study at SATVI site: TruCulture at weeks 0, 16, 24

Comparison with QFT, flow cytometry, mRNA biomarkers, PET/CT, urine, saliva

Chen et al, Gates Open Research 2017
Understanding immune variability for new clinical strategies

How are immune responses different in disease?

How do immune responses differ with age, sex, genetics & environment?

What are the cellular mechanisms of these differences?

Tuberculosis
Diagnosis
Treatment
Vaccination
Hypothesis Testing: does variability in pre-vaccine immunity associate with post-vaccine responsiveness?

Pre-Vaccine Innate immunity

- TLR7 (mRNA vaccine)
- TLR9 (viral vector)

Pfizer BNT162 v Sinovac CoronaVac vaccination

Adolescents & Parents (Hong Kong University/C2i)

Post-Vaccine Adaptive immunity

- Blood & nasal IgG/IgA
- Blood T/B cells
- SARS-CoV-2 Spike
Studying variable vaccine induced immunity - Influenza

Hypothesis Testing: does variability in pre-vaccine immunity associate with post-vaccine responsiveness?

**Vaccination**

**Pre-Vaccine Innate immunity**
- IFN-I
- TLR3
- TLR7
- TLR9
- T cell responses

**Seasonal Influenza vaccine**
- Healthy adult donors
  - European site
  - Indian site

**Post-Vaccine Adaptive immunity**
- Blood IgG
- Blood T/B cells
Identification of genetic determinants of BCG immunity

1,000 healthy donors

BCG stimulation – can we identify genetic determinants of immune responses?

Deteminants of immune variability

Null control

BCG stimulation

Baseline QTL

Response QTL

Piasecka B, Duffy D et al PNAS 2017
Mapping Quantitative Trait Loci (eQTL) identifies novel genetic associations

Deteminants of immune variability

Genetic variant present in Europeans but not African populations

TLR 10/1/6 genetic locus

Pro-inflammatory (e.g., IL18, TLRs,)

Anti-inflammatory (e.g., IL-10, TGFB, LILR)

Homozygous major

Heterozygous

Homozygous minor

Quach et al Cell 2016
The ongoing evolution of Milieu Intérieur

Extending the MI approach to more integration, deeper phenotyping, other ages & populations

- Compare population level aging effects with individual level variation
- Assess immune variability in other key stages of life & other populations

Key stages:
- Karolinska: 0-2 yrs
- ATRACTion (Imagine): 2-8 yrs
- TILDA (Trinity): >75 yrs
- Other populations: Pasteur Network, Hong Kong & Senegal