Bringing mass cytometry to the clinic

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Disclosures

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Fluorescence versus mass cytometry
Fluorescence versus mass cytometry

**Fluorescence cytometry**

- **CD4**
- **CD8**
- **PE**
- **PE/CF594**
Fluorescence versus mass cytometry

Fluorescence cytometry

MASS cytometry

Cytometry by Time of Flight mass spectroscopy (CyTOF)
Lanthanide metals for mass cytometry

- Abundant tags of similar intensity (particularly between 159-170)
- Discrete signals: minimal overlap and predictable (fewer controls to run)
- Zero background cellular signal
Advantages of mass cytometry

- Rare/limited samples: need to address as many questions as possible
  - precious samples from patients
- Extra information from combinatorial staining
  - polyfunctional cytokine production, co-expression of >10 markers on a single cell type
- Lack of biological background
- Absence of autofluorescence
  - can fix and store processed samples for extended periods at room temperature
  - especially useful for clinical samples from peripheral sites
Disadvantages of mass cytometry

- No sorting capacity – sample is destroyed by analysis
- Slow rate of data acquisition
  - 1000 cells/sec vs 20,000 cells/sec for fluorescence flow cytometry
- Signals are lower than the brightest fluorochromes such as phycoerythrin, but similar to the majority of fluorochromes
Mass cytometry experiment design

- Barcoding and pooling of samples

1. Barcoding antibody (CD45)
2. Cocktail of surface antibodies
5. Differential signal detected
Mass cytometry experiment design

• Barcoding and pooling of samples

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Sample 1
Sample 2

• Post acquisition data processing and QC

- FCS3.0 files acquired

Helios
- Data normalised according to EQ Four Element Calibration Beads

Matlab
- Files debarcoded by CD45 barcoding antibody signals
- Files concatenated if sample acquisition was interrupted

Flowjo
- Live cell events exported and loaded in new workspace
- Immune populations gated for each sample

Flowjo
- QC and conversion of population quantity to $x10^9$/L metric
- tSNE and PCA analysis

Pipeline script
Recapitulation of population hierarchies

- Comprehensive panel design
- Using the biology we know to gate canonical immune populations
Immune signatures in autoimmune disease

Psoriasis

Rheumatoid arthritis
Mass cytometric analysis of PBMC from advanced melanoma patients
Mass cytometric analysis of PBMC from advanced melanoma patients

**Non-responders** – Patients whose melanoma progressed on anti-PD-1 therapy

**Responders** – Patients with a complete or partial response, or stable disease, on anti-PD-1 therapy

**Adverse event** – Patients who experienced an adverse event requiring cessation of anti-PD-1 therapy

**Controls** – Healthy subjects, age- and sex-matched with patients

2 samples per patient – one before the first dose of therapy, and one before the second dose 2-3 weeks later.

1 sample for each age and sex matched control

Each data point represents a cell subset

Data are row normalised to compare relative change in each cell subset, not absolute number.
Mass cytometric analysis of PBMC from advanced melanoma patients

SAM analysis reveals no significant differences between responders, controls, and patients who suffered adverse events.
Mass cytometric analysis of PBMC from advanced NSCLC patients
Imaging mass cytometry (IMC)
‘Hyperion’ system from Fluidigm
Imaging mass cytometry

**Figure 1** | Workflow of imaging mass cytometry.

*Giesen et al, 2014, Nature Methods*
Similar to standard immunofluorescence staining – except in 40 dimensions

**Figure 3.** IMC patterns for normal human testis, kidney, and prostate, obtained from a tissue microarray. The total ion current for each organ is shown in the left columns, and the RGB images are based on combinations of individual antibodies imaged by IMC, chosen to highlight normal anatomical features of each organ. [Color figure can be viewed at wileyonlinelibrary.com]

Chang et al, 2017, Cytometry A
Data processed to single cells: *Sydney based imaging mass cytometry*

Human Spleen

‘Segmentation’ with Histocat software
Summary

• Mass cytometry and mass imaging have great potential to provide another dimension to our understanding of human disease states, particularly those that involve immune responses and inflammation

• Analysis of the distribution of blood cell subsets can define an immune signature characteristic of each individual

• Immune signatures can predict response to cancer immunotherapy
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