Development of a 37-Channel Mass Cytometry (CyTOF) Panel to Predict Treatment Response in Rheumatoid Arthritis

Document Version
Final published version

Link to publication record in Manchester Research Explorer

Citation for published version (APA):

Published in:
European Medical Journal Rheumatology

Citing this paper
Please note that where the full-text provided on Manchester Research Explorer is the Author Accepted Manuscript or Proof version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version.

General rights
Copyright and moral rights for the publications made accessible in the Research Explorer are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Takedown policy
If you believe that this document breaches copyright please refer to the University of Manchester’s Takedown Procedures [http://man.ac.uk/04Y6Bo] or contact uml.scholarlycommunications@manchester.ac.uk providing relevant details, so we can investigate your claim.
DEVELOPMENT OF A 37-CHANNEL MASS CYTOMETRY (CYTOF) PANEL TO PREDICT TREATMENT RESPONSE IN RHEUMATOID ARTHRITIS

*Ben Mulhearn,1,2 Sebastien Viatte,2 Tracy Hussell,1 Anne Barton1,3

1. Manchester Collaborative Centre for Inflammation Research (MCCIR), University of Manchester, Manchester, UK
2. Division of Musculoskeletal and Dermatological Sciences, University of Manchester, Manchester, UK
3. Kellgren Centre for Rheumatology, Central Manchester Hospitals NHS Foundation Trust, Manchester, UK

Correspondence to ben.mulhearn@manchester.ac.uk

Disclosure: MCCIR is part-funded by both AstraZeneca and GlaxoSmithKline.

Citation: EMJ Rheumatol. 2017;4[1]:XX-XX. Abstract Review No. ARXX.

Keywords: Cytometry using time-of-flight mass spectrometry (CyTOF), cluster identification, characterisation, and regression (CITRUS), T cell, proteome, immunology, biologic drugs, rheumatology.

Simultaneously, advances in non-biased clustering algorithms and their accessibility has allowed multidimensional datasets to be analysed much more comprehensively.

This project optimised a CyTOF T cell panel and tested a novel non-biased clustering algorithm against conventional biaxial gating. Ten healthy controls (HC) and 10 RA patients were included. T cells were stimulated for 4 hours using anti-CD3/anti-CD28 beads, then stained with a 37-channel mass cytometry panel including surface markers, intracellular antigens, and transcription factors (Figure 1). Analysis was performed by biaxial gating and by cluster identification, characterisation, and regression (CITRUS). The CITRUS algorithm compared the two groups: stimulated HC versus stimulated RA T cells. To compare abundances of cell clusters, the predictive association model ‘Prediction Analysis for Microarrays in R’ was used with a minimum cluster size of 2.2%, five cross-validation folds, and a false discovery rate of 1%

Conventional gating showed that cytokine expression was highly variable within and between HC and RA samples, but no significant differences were found when comparing HC and RA samples. However, using CITRUS we identified three clusters of cells which were significantly different in abundance between HC and RA. Cluster 1 was CD4+CD8+, had characteristics of regulatory T cells, and was less abundant in RA. Cluster 2 was CD28−CD8+ expressing perforin and Tbet, and Cluster 3 was a CD4+CD8+CD127+CCR6+ population, both of which were more abundant in RA.

To conclude, although cytokine expression of ex vivo stimulated T cells from RA and HC is highly variable, no differences were found between the two groups with conventional gating and this may have been due to small sample numbers. Using the fully automated clustering algorithm CITRUS, differences in the abundance of three novel cell clusters between the RA and HC groups were revealed and the phenotype of these clusters ascertained. These clusters would have been missed using biaxial gating alone.

Our next step is to compare RA responders to non-responders using an automatic clustering algorithm. By doing so, novel cell clusters might be identified which differ between responders and non-responders, and may go some way to predict treatment response. Furthermore, the function of novel clusters might be further characterised by isolation using fluorescence-activated cell sorting and functional assays. Finally, although in this study biaxial gating did not reveal any significant differences, we believe that automatic clustering algorithms should be used in conjunction with conventional gating when analysing high-dimensional datasets to maximise the scientific output of the data.

REFERENCES

Figure 1: CyTOF T cell panel.

HLA-DR: human leukocyte antigen-D related; IFN-γ: interferon gamma; IL: interleukin; PD-1: programmed cell death protein 1; TNF: tumour necrosis factor.