



UNIVERSITY OF LEEDS

This is a repository copy of *A2.40 Investigating IL-6 pathway signalling kinetics in peripheral blood single cell subsets with tocilizumab therapy in patients with early rheumatoid arthritis*.

White Rose Research Online URL for this paper:  
<http://eprints.whiterose.ac.uk/119937/>

Version: Accepted Version

---

**Proceedings Paper:**

Ouboussad, L, Wong, C, Hunt, L et al. (3 more authors) (2016) A2.40 Investigating IL-6 pathway signalling kinetics in peripheral blood single cell subsets with tocilizumab therapy in patients with early rheumatoid arthritis. In: *Annals of the Rheumatic Diseases*. 36th European Workshop for Rheumatology Research, 25-27 Feb 2016, York, UK. BMJ Publishing Group , A31-A31.

<https://doi.org/10.1136/annrheumdis-2016-209124.75>

---

© 2016, Published by the BMJ Publishing Group Limited. This is an author produced version of a paper published in *Annals of the Rheumatic Diseases*. Uploaded in accordance with the publisher's self-archiving policy.

**Reuse**

Unless indicated otherwise, fulltext items are protected by copyright with all rights reserved. The copyright exception in section 29 of the Copyright, Designs and Patents Act 1988 allows the making of a single copy solely for the purpose of non-commercial research or private study within the limits of fair dealing. The publisher or other rights-holder may allow further reproduction and re-use of this version - refer to the White Rose Research Online record for this item. Where records identify the publisher as the copyright holder, users can verify any specific terms of use on the publisher's website.

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.



[eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk)  
<https://eprints.whiterose.ac.uk/>

## **Investigating IL-6 pathway signalling kinetics in peripheral blood single cell subsets with tocilizumab therapy in patients with early rheumatoid arthritis**

Lylia Ouboussad<sup>1,2</sup>, Chi Wong<sup>1,2</sup>, Laura Hunt<sup>1,2</sup>, Paul Emery<sup>1,2</sup>, Michael F. McDermott<sup>1,2</sup>, Maya H. Buch<sup>1,2</sup>

<sup>1</sup>Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK; <sup>2</sup>NIHR-Leeds Musculoskeletal Biomedical Research Unit (NIHR-LMBRU), Chapel Allerton Hospital, Leeds, UK.

### **Background and objectives**

Rheumatoid arthritis (RA) arises in individuals with a genetic predisposition, triggered by environmental influences, leading to dysfunctional immune responses. The importance of pro-inflammatory cytokines, such as TNF and IL-6, in RA is well recognised, and the successful use of biologic agents inhibiting their action is widely established. The study of the intracellular effect of cytokine ligation to their receptors is of interest in elucidating mechanisms of action and potentially response prediction.

This project focuses on the IL-6 signalling pathway and its blockade, using tocilizumab (TCZ; IL-6 receptor monoclonal antibody) to (i) determine the relative roles of the 3 arms of the pathway (JAK-STAT but also, PI3K/Akt and MAPK/ERK) in T-cells, B-cells and monocytes; (ii) examine whether there is heterogeneity in the predominant IL-6 intracellular signalling pathway and whether this associates with response to TCZ (iii) the effect of TCZ therapy on intracellular pathways.

### **Materials and methods**

Multiparameter phosflow cytometry method to identify phosphorylation intensities of transcription factor STAT3 and tyrosine kinases Akt and Erk that cover the entire IL-6 pathway is being undertaken. Twenty patients with treatment-naïve, early RA; 10 of whom are receiving TCZ monotherapy and 10 receiving combined methotrexate and TCZ have been recruited. Peripheral blood mononuclear cells (PBMCs) have been isolated at baseline, weeks 24 and 48 after treatment and cryopreserved. Healthy individual samples have been used as control. PBMC are either unstimulated, or stimulated with IL-6 or PMA, in order to activate the pathway. Median fluorescence intensity (MFI) is being measured using LSRII (BD Biosciences), and data are analysed using BD FACSDiva software.

### **Results**

After adequate optimisation and using a gating strategy to identify immune cell subsets, including lymphocytes (T, B and NK cells) and monocytes (CD14 and CD16 subsets), the phosphorylation kinetics of p-STAT3, p-Akt, p-Erk1/2 are being monitored. Preliminary data suggest that there are differences in immune cell signalling between healthy individuals and RA patients. In addition, following stimulation, differences in the MFI have been observed in the cell subsets. The phosflow cytometry and data analyses will be completed shortly and presented as heat maps to illustrate baseline differences and changes following TCZ.

## **Conclusions**

Comprehensive evaluation of IL-6 intracellular signalling within immune cells from RA patients will provide insights into disease pathophysiology and heterogeneity, TCZ drug mechanism of action and possibly prediction of outcome/response.