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

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## 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.