

PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form ([see an example](#)) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below. Some articles will have been accepted based in part or entirely on reviews undertaken for other BMJ Group journals. These will be reproduced where possible.

ARTICLE DETAILS

TITLE (PROVISIONAL)	IL-6 pathway–driven investigation of response to IL-6 receptor inhibition in rheumatoid arthritis
AUTHORS	Harari, Olivier; Wang, Jianmei; Platt, Adam; Upmanyu, Ruchi; Germer, Søren; Lei, Guiyuan; Rabe, Christina; Benayed, Ryma; Kenwright, Andy; Hemmings, Andrew; Martin, Mitchell

VERSION 1 - REVIEW

REVIEWER	Jessop, David University of Bristol, Laboratories of Integrative Neurosciences No competing interests to declare
REVIEW RETURNED	15-May-2013

GENERAL COMMENTS	<p>This study is designed to determine whether individual alterations in the IL-6 pathway in patients with RA are associated with variability in clinical responsiveness.</p> <p>The study benefits from a large cohort of patients. The results as expressed as correlations between measurements of disease activity and parameters of the IL-6 pathway are largely negative. One possible reason for this is that both disease activity and IL-6 have pronounced circadian variation. RA is characterized by early morning joint stiffness (EMS) and associated high DAS while serum IL-6 levels rise throughout the night prior to onset of EMS (see papers by Cutolo; Chrousos etc). If blood samples are not taken during a well-defined period during the rise of IL-6, no meaningful correlation can be made between IL-6 and EMS. There is no evidence in this paper that this was done and given the large number of samples from multiple clinics, it is probable that blood samples were taken at many different times of the day.</p> <p>In this paper, baseline serum IL-6 is elevated in RA patients, an observation which is consistent with many reports in the literature in smaller studies. However, why this pro-inflammatory cytokine should be further elevated in patients showing clinical improvement to tocilizumab is difficult to explain and indeed the authors do not attempt this in the Discussion. It is possible that this anomaly is due to the above-mentioned lack of correlation between disease measurement and blood sampling times.</p> <p>In fact, it has been demonstrated by Buttgerit et al. (Lancet 2008) and Kirwan et al (Ann NY Acad Sci 2010) that clinical improvement in RA in response to timed release of prednisone is associated with significantly decreased serum IL-6. In the context of these papers, the statement in the Results that 'baseline serum IL-6 level is not a general prognostic factor for clinical response in RA' is quite wrong. Neither the work of Buttgerit or Kirwan is cited and the authors should re-evaluate their data in light of these observations in the</p>
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	<p>literature.</p> <p>One further comment: it should be stated that RA patients in the study were free of glucocorticoids for 3 months prior to the study (if this is indeed the case).</p>
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REVIEWER	Morgan, Ann University of Leeds, Leeds Institute of Molecular Medicine
REVIEW RETURNED	05-Jun-2013

THE STUDY	This is a sound manuscript and worthy of publication. I've made a number of relatively minor comments that in my opinion would enhance the scientific value of the manuscript.
GENERAL COMMENTS	<p>Personalised/ stratified medicine is an important goal for the treatment of RA and Roche are to be congratulated for undertaking this work and making the data generated publically available. The data from 5 clinical trials where tocilizumab has been used in either the 4 or 8mg/kg dose are collated and presented in this manuscript that explores serum, genetic and genomic biomarkers within the IL-6 pathway as predictors of response to tocilizumab in RA. The manuscript is well written and the supplementary data are comprehensive and will support future meta-analyses as academic groups also collate data from patients treated in routine clinical practice.</p> <p>I have a few comments with respect to this manuscript: It would be helpful to include the number of cases analysed in the abstract to this paper. Given the ultimate goal of stratified medicine and effect of IL-6 on B-cell proliferation and antibody production, it would be interesting to determine whether there was a difference in IL-6 serum levels between the autoantibody positive and negative cohorts and whether a difference in predictive ability of the IL-6 biomarkers was observed between these two RA populations. All sections of the manuscript could be updated to consider whether autoantibodies influence outcome, including any data from the clinical trials to support this. The ethnicity of the patients recruited to the different studies should be presented, including statistical methods to control for ethnicity in the analyses, particularly if a substantial number of individuals were recruited from non-European populations. Were principle component analyses performed/ ancestry markers included in the analyses of the total patient cohort, for example. The method(s) of DNA and RNA extraction should be stated and although the QC for the genotyping and transcript studies were included in the supplementary material, these should be cross-referenced in the genotyping section of the methods. A brief statement about the genotyping success rate would be helpful in the methods. It would be helpful to expand on how the genes contained in the IL-6 pathway and network were selected. For the Tier 1 markers how were the IL-6 and IL-6R regions defined and how much coverage of these genes was provided by the variants on the Genechip array. The first mention of the IL-6ST is in the methods section and the role of this protein in IL-6 signalling should be clearer in the introduction. The power of the studies should be provided, particularly for the inclusion of such a large number of disease activity measures. How was the shared epitope genotyped? Was this imputed from the</p>

	<p>array or an alternative genotyping assay performed. Details should be provided in the methods. Did the shared epitope predict treatment response?</p> <p>Page 14, results. It would be helpful to state what the 3 populations examined refer to at the start of the results section.</p> <p>For the analyses of IL-6 serum levels and clinical response was there any evidence that serum IL-6 performed better than CRP itself in predicting cDAS28</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer #1: David Jessop

Comment #1 The study benefits from a large cohort of patients. The results as expressed as correlations between measurements of disease activity and parameters of the IL-6 pathway are largely negative. One possible reason for this is that both disease activity and IL-6 have pronounced circadian variation. RA is characterized by early morning joint stiffness (EMS) and associated high DAS while serum IL-6 levels rise throughout the night prior to onset of EMS (see papers by Cutolo; Chrousos etc). If blood samples are not taken during a well-defined period during the rise of IL-6, no meaningful correlation can be made between IL-6 and EMS. There is no evidence in this paper that this was done and given the large number of samples from multiple clinics, it is probable that blood samples were taken at many different times of the day. The study protocols specified that baseline IL-6 samples must be collected prior to tocilizumab infusion and fasting. Exact sampling times are available and have been added to the Methods section and as Supplementary Figure 2. Limitations due to variable sampling times have been assessed in the discussion along with the reference on the effect of circadian rhythm on IL-6 (Kirwan JR et al. *Ann N Y Acad Sci* 2010;1193:127-33).

Comment #2 In this paper, baseline serum IL-6 is elevated in RA patients, an observation which is consistent with many reports in the literature in smaller studies. However, why this pro-inflammatory cytokine should be further elevated in patients showing clinical improvement to tocilizumab is difficult to explain and indeed the authors do not attempt this in the Discussion. It is possible that this anomaly is due to the above-mentioned lack of correlation between disease measurement and blood sampling times. Increased activity in the IL-6 pathway is a hypothetical reason for an inhibitor to that pathway to have an enhanced response, as mentioned in the Introduction and cited in the Discussion (Reference #26; Littman BH. *Tocilizumab and missed personalized medicine opportunities for patients with rheumatoid arthritis? Arthritis Rheum* 2009;60:1565-66). Therefore no changes have been made based on this comment.

Comment #3 In fact, it has been demonstrated by Buttgerit et al. (*Lancet* 2008) and Kirwan et al (*Ann NY Acad Sci* 2010) that clinical improvement in RA in response to timed release of prednisone is associated with significantly decreased serum IL-6. In the context of these papers, the statement in the Results that 'baseline serum IL-6 level is not a general prognostic factor for clinical response in RA' is quite wrong. Neither the work of Buttgerit or Kirwan is cited and the authors should re-evaluate their data in light of these observations in the literature. The reference cited only demonstrated that IL-6 levels were reduced by effective treatment. It did not indicate an association between baseline IL-6 and treatment response. To our knowledge, there has not been any evidence that baseline IL-6 alone is a predictor of clinical response. To further clarify, we revised our statement in the Result section and added comments in the Discussion.

Comment #4 It should be stated that RA patients in the study were free of glucocorticoids for 3 months prior to the study (if this is indeed the case). In all studies, oral corticosteroids (≤ 10 mg/day prednisone or equivalent) were permitted if the dose was stable for at least 6 weeks prior to baseline. Clarification has been added to the Methods text.

Reviewer #2: Ann Morgan

Comment #1 It would be helpful to include the number of cases analysed in the abstract to this paper. Number of analyses has been added to abstract.

Comment #2 Given the ultimate goal of stratified medicine and effect of IL-6 on B-cell proliferation and antibody production, it would be interesting to determine whether there was a difference in IL-6 serum levels between the autoantibody positive and negative cohorts and whether a difference in predictive ability of the IL-6 biomarkers was observed between these two RA populations.

All sections of the manuscript could be updated to consider whether autoantibodies influence outcome, including any data from the clinical trials to support this. Rheumatoid factor (RF) was measured in all studies, and we have added results as outlined below. Anti-CCP was not measured except for a very small number of patients; therefore, we have not added.

We have added the additional analysis of baseline IL-6 vs. RF in the Results section. We also added results of baseline IL-6 vs. clinical response in the RF-positive subpopulation. The RF-negative subpopulation is too small for the association analysis.

Comment #3 The ethnicity of the patients recruited to the different studies should be presented, including statistical methods to control for ethnicity in the analyses, particularly if a substantial number of individuals were recruited from non-European populations. Were principle component analyses performed/ ancestry markers included in the analyses of the total patient cohort, for example. The authors recognise that there is genetic diversity in the patient population. In Table 1, we showed the percentage of White (European descent) patients for the DNA analysis population. Overall, 79% of all patients are White. This is now mentioned in the Results section. Because the current manuscript addresses the effect of target genes, the genome-wide association study (GWAS) approach of adjusting for the principal component analysis (PCA) was not conducted. To reduce the effect of genetic diversity, all statistical analyses of the DNA markers were repeated in the White subpopulation, and the population was indicated in the Results presented within Table 3 and Table 4. Further comments on the ethnicity issue have also been added to the Discussion section.

Comment #4 The method(s) of DNA and RNA extraction should be stated and although the QC for the genotyping and transcript studies were included in the supplementary material, these should be cross-referenced in the genotyping section of the methods. A brief statement about the genotyping success rate would be helpful in the methods. DNA/RNA extraction methods were added to the supplementary material and referenced in the main text.

Cross-reference to the supplementary material describing QC methods for genotyping and transcript studies is now included in the Genotyping section of the Methods as well as the Transcript Analysis section and the Statistical Analysis Section. In addition, we mentioned the threshold of 95% for the call rate.

Comment #5 It would be helpful to expand on how the genes contained in the IL-6 pathway and network were selected. Expanded method description has been added at the beginning of the supplementary material.

Comment #6 For the Tier 1 markers how were the IL-6 and IL-6R regions defined and how much coverage of these genes was provided by the variants on the Genechip array. Definition of regions and coverage has been added to the Genotyping section of Methods.

Comment #7 The first mention of the IL-6ST is in the methods section and the role of this protein in IL-6 signalling should be clearer in the introduction. IL-6ST has been defined in the Introduction.

Comment #8 The power of the studies should be provided, particularly for the inclusion of such a large number of disease activity measures. An explanation of power has been added to the Discussion.

Comment #9 How was the shared epitope genotyped? Was this imputed from the array or an alternative genotyping assay performed. Details should be provided in the methods. Did the shared epitope predict treatment response? A brief description of shared epitope genotyping has been added at the end of the Genotyping section in Methods. The shared epitope was used as only a covariate in the genetic analysis and was not the focus of this investigation; therefore, only brief methods have been provided. Detailed methods can be found in other publications from the group who provided these data, such as <http://onlinelibrary.wiley.com/doi/10.1002/art.10485/full>

We used the shared epitope only as a covariate in the genetic analysis. It is not within the scope of

this manuscript to investigate the effect of shared epitope. We have unpublished data showing that the number of shared epitope alleles or shared epitope genotypes have no clinically significant effect on treatment response to tocilizumab.

Comment #10 Page 14, results. It would be helpful to state what the 3 populations examined refer to at the start of the results section. The DNA, RNA and serum sample subgroups have been defined at the beginning of the Results section with a subheading and a description of baseline characteristics.

Comment #11 For the analyses of IL-6 serum levels and clinical response was there any evidence that serum IL-6 performed better than CRP itself in predicting cDAS28 Our unpublished data show that the strength of the association was very similar between IL-6 vs. clinical response and CRP vs. clinical response. Given that CRP was used as a response endpoint in this analysis, using baseline CRP as a predictor is beyond the scope of this manuscript. The association of CRP with clinical response in phase 3 studies was published in a poster at EULAR 2011 (Emery P et al. Relationship between pre-treatment CRP and clinical efficacy following treatment with tocilizumab: results from a pooled analysis of tocilizumab phase 3 studies).

VERSION 2 – REVIEW

REVIEWER	Ann Morgan, University of Leeds No conflicts of interest
REVIEW RETURNED	24-Jul-2013

- The reviewer completed the checklist but made no further comments.