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Md Yusof, MY orcid.org/0000-0003-3131-9121, Kabia, A, Darby, M et al. (5 more authors) (2017) Effect of rituximab on the progression of rheumatoid arthritis–related interstitial lung disease: 10 years' experience at a single centre. Rheumatology, 56 (8). pp. 1348-1357. ISSN 1462-0324

https://doi.org/10.1093/rheumatology/kex072

Effect of rituximab on the progression of rheumatoid arthritis-related interstitial lung disease: 10 years' experience at a single centre

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ABSTRACT

Objective: To evaluate the effect of rituximab in patients with rheumatoid arthritis-related interstitial lung disease (RA-ILD) and identify factors associated with outcome after treatment.

Methods: An observational study of patients with RA-ILD was conducted from a cohort of rituximab treated RA patients in a single centre for over 10 years. Progression was defined by any of the following: a decrease of pre-rituximab FVC>10% or DLCO>15% predicted; worsening of ILD score (ILDS); or death from progressive ILD.

Results: Of 700 RA patients treated with rituximab, 56 had RA-ILD (prevalence=8%). After rituximab, new ILD was diagnosed in 3/700 patients (incidence=0.4%). Data for lung assessment was available for 44/56 patients. The median relative change pre- and post-rituximab for FVC were -2.4% and +1.2%; p=0.025 and for DLCO were -4.4% and -1.3%; p=0.045. Post-rituximab, 23/44(52%) were stable and 7/44(16%) had improved. Of the 14(32%) with ILD that progressed, 9/56(16%) were deaths due to progressive ILD. Factors associated with ILD progression were radiologic pattern of usual interstitial pneumonia, a previous history of lung progression and pre-rituximab DLCO<46% predicted. Of those whose ILD progressed, 11/14(79%) had severe ILD before rituximab (median DLCO=42% predicted (IQR 41-49).

Conclusion: In this cohort of patients where rituximab was given for arthritis, most patients with ILD pre-rituximab remained stable/improved after treatment over a prolonged follow-up period. Patients who deteriorated/died had the most severe ILD pre-rituximab, suggesting the drug was not contributory. Rituximab appears to be an acceptable therapeutic choice for patients with RA-ILD and further studies are warranted.

Key words: B cells, Biological Therapies, Immunosuppressant, Respiratory, Rheumatoid arthritis

Key Messages:

1. Rituximab showed satisfactory safety in rheumatoid arthritis-related interstitial lung disease.
2. Lung function remained stable/improved in most patients after rituximab over a prolonged follow-up period.
3. Usual interstitial pneumonia, previous progression and low carbon monoxide diffusing capacity predicted lung progression post-rituximab.

INTRODUCTION

Interstitial lung disease (ILD) is a common extra-articular manifestation of rheumatoid arthritis (RA) that is reported in up to 30% of RA patients (1). RA-ILD is the second most common cause of mortality in RA (2). The high mortality has been attributed to uncontrolled systemic inflammatory disease, infections and complication from therapies (3, 4). While the treatment of RA has greatly improved in recent years with the introduction of biological therapies, the use of such agents has often been restricted in RA-ILD due to concerns over safety.

Initial concerns arose after anecdotal reports of serious respiratory adverse events following treatment with a TNF-inhibitor (TNF-i) in patients with pre-existing RA-ILD (5, 6), leading to preference for a non-TNF-i such as rituximab. Histologically, a rationale for B-cell targeted therapy in ILD was suggested by the demonstration of follicular B-cell hyperplasia and interstitial plasma cell infiltrates in (open) lung biopsy specimens of patients with RA-ILD compared to idiopathic pulmonary fibrosis (7). Nevertheless, clinical evidence for the efficacy and safety of rituximab in the context of ILD was scarce. Indeed, in the only prospective pilot study of 10 patients with progressive RA-ILD who were treated with rituximab, the association between significant adverse events (included two deaths) and either rituximab or underlying

disease could not be dissected due to the small number of patients (8). Furthermore, patients with RA-ILD are normally excluded from formal clinical trials due to comorbidity. Therefore, data from larger cohorts and registries are needed.

In the absence of a head-to-head trial of rituximab against a standard therapy and/or other biologics, the aims of this study were: to evaluate the effect of rituximab in patients with RA-ILD as assessed using pulmonary function test (PFT), imaging and mortality; and to identify factors associated with outcome post-rituximab.

METHODS

Patients and Design

All patients with moderate to severe RA who were treated with rituximab in our unit between January 2004 and May 2015 were evaluated retrospectively from the Leeds Biologics Database. From this, an observational study of consecutive patients with RA-ILD was conducted. Inclusion criteria included adults (>18 years old), fulfilling the revised 1987 American College of Rheumatology for RA (9) and detection of ILD by high resolution computer tomography (HRCT). Leeds (West) Research Ethics Committee confirmed that ethical approval was not required in accordance with the UK National Health Service Research Ethics Committee guidelines because all treatment decisions were made prior to evaluation of data.

Treatment Protocol

All patients received a first cycle of therapy consisted of 100 mg of methylprednisolone and 1000 mg of rituximab given intravenously on days 1 and 14. Further cycles consisted of the same regimen were repeated on clinical relapse. Rescue therapies with intravenous cyclophosphamide and/or referral for lung transplantation could be undertaken in the event of lung progression/worsening (as defined below).

Clinical Data and Outcomes

Joints

Disease activity was assessed using Disease Activity Score in 28 joints (DAS-28) at baseline and every 3 months. Response at 6 months was defined according to the criteria of the European League Against Rheumatism (EULAR) (10).

Lung

Data from PFT; consisting of assessment for forced vital capacity (FVC) and carbon monoxide diffusing capacity (DLCO), was taken at 6-12 months pre-rituximab, at the time of treatment with rituximab, 6-12 months post-rituximab and from most recent follow-up.

HRCT scans were acquired (when clinically indicated) in patients with worsening dyspnoea and/or deterioration in lung function using a standardised method. The scans were scored independently by two radiologists; MD – a chest radiologist with over 10 years' experience in reporting ILD and GL – a general radiologist; both blinded to lung function information and the sequence of scans. ILD Score (ILDS) was used to evaluate the presence and extent of pure ground glass opacification (GGO), pulmonary fibrosis (PF) and honeycombing (HC) in the six lung zones; with a maximum total score possible of 24 (11). Each paired scan (pre- and post-treatment) was then rated as 0=worsening, 1=same and 2=improving. Any discrepancy was resolved by consensus. Details methodology for HRCT scans can be found in Supplementary file, available at Rheumatology online.

In order to account for missing PFT data of those with severe ILD who were unable to perform the test, data from HRCT and survival status were incorporated into the overall lung response. This lung response was classified into: worsening = any of either a decrease of pre-rituximab FVC>10% or DLCO>15% predicted, worsening of ILDS or death from progressive lung (12); improving = any of either an increase of pre-rituximab FVC>10% or DLCO>15% predicted or improvement of ILDS; and stable = others which did not meet criteria for either worsening/improving.

Peripheral blood B-cell subsets analysis

Peripheral blood B-cell subsets were analysed using highly sensitive flow cytometry (HSFC) as previously described (13) at week 0, 2 and 26 without knowledge of clinical status other than time since rituximab. Complete B-cell depletion was defined as counts $<0.0001\times10^9$ cells/L.

Safety

Safety assessments which included severe adverse events (SAEs) and serious infection were recorded irrespective of possible association with RA-ILD and or therapy. SAEs were defined as those resulting in either hospitalisation that lasted more than 24 hours, flares requiring intravenous therapy, malignancies, life-threatening situations or death. Data for serious infections was gathered from hospital admission records using Patient Access Centre (PAS) system and was later confirmed with case notes.

Statistical Analysis

Pulmonary function trends were expressed as relative change from start of therapy with rituximab, and Wilcoxon signed-rank test was used to analyse pulmonary function changes before and after treatment. The difference in clinical characteristics between RA-ILD patients who had lung progression versus those who were stable post-rituximab were analysed using Mann-Whitney U test for continuous variables and Fisher's Exact test for categorical variables accordingly.

Progression-free survival time (measured in weeks) was calculated from the date of first rituximab infusion to either the date of progression or the date of data last updated (May 2015). Analysis for categorically distributed variables that were relevant for ILD progression was calculated using Kaplan–Meier plot and log-rank test. Multivariate analysis was not performed due to the number of patients and 9 potential predictors of ILD progression (14). Receiver operator curves (ROC) were used to measure the sensitivity and specificity of optimal thresholds for investigations predicting ILD progression. All statistical analysis was performed using SPSS 21.0 and Graph Pad Prism 7.01 for Windows.

RESULTS

Patient Characteristics

Of 700 patients with RA treated with rituximab, 56 patients had RA-ILD (prevalence = 8%) and were included in the analysis. Thirty-six were female; 55/56 (98%) were rheumatoid factor and/or anti-cyclic citrullinated peptide antibody positive, median age 64 years (IQR 59-72);

median RA duration 10 years (IQR 7-13); median ILD duration 5 years (IQR 3-7); median FVC 87% predicted (IQR 76-108) and median DLCO 58% predicted (IQR 43-63) at rituximab initiation. Total follow-up: 195 patient-years. Baseline characteristics are described in Table 1. Post-rituximab, new ILD was diagnosed in only 3/700 patients (incidence = 0.4%).

Treatment Characteristics

A hundred and eighty one cycles of rituximab were administered to the 56 patients studied. Median (IQR) duration of response for cycles 1–3 (C1–3) were 44 (33–55), 44 (37–58) and 43 (35–66) weeks respectively. Prior to C1, 16 were treated previously with a TNF-i. Of these, 10 patients (63%) were switched to rituximab due to worsening ILD while 6 (37%) had secondary non-response in terms of RA. In C1, 36 patients (64%) received concomitant therapies with conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs): methotrexate=28, azathioprine=5, leflunomide=2 and mycophenolate mofetil=1.

Ten patients received cyclophosphamide prior to rituximab. Of these, 7 had stable ILD during rituximab treatment while 3 required further cyclophosphamide due to ILD progression. Two patients who had not previously received cyclophosphamide received the treatment post-rituximab due to worsening ILD.

Articular Response

In C1, there was a significant reduction in DAS-28; mean pre-rituximab 5.69 versus 4.07 post-rituximab, mean difference -1.62 ± 0.29 (95% confidence interval (CI) -2.20 to -1.05); $p<0.001$. EULAR response rates; Good, Moderate and Poor in patients with complete data at 6 months post-rituximab were 12/52 (23%), 32/52 (62%) and 8/52 (15%) respectively. Articular response was not correlated with lung response; r=0.122 (95% CI -0.206 to 0.426); p=0.452.

Of the 8 patients who were C1 non-responders, 7/8 had incomplete B-cell depletion post-rituximab. Six of these were re-treated at 6 months with depletion in 3 patients but all responded in C2. 1/8 of the C1 non-responders had ILD progression post-rituximab. The response rates (EULAR Good and Moderate) for C2 and C3 were 32/40 (80%) and 23/30 (78%) respectively. At the last follow-up, 7 (13%) had secondary non-response to rituximab and were switched to different biologics; tocilizumab (n=5) and abatacept (n=2).

In C1, 23/56 (41%) were on concomitant corticosteroid at rituximab initiation. At 6 months post-rituximab, cessation of corticosteroid was achieved in 3/23 (13%), 4/23 (17%) had dose reduction of more than 50% from baseline, 14/23 (61%) had their dose unchanged and 2/23 (9%) had their dose increased by 50% from baseline.

Lung Response

Data for the overall lung assessment was available for 44/56 patients. Of these, pre- and post-rituximab PFT results were recorded in 37/44. The remaining 7/44 had death outcome reported only as they were unable to undergo either a PFT or HRCT.

PFT

In the 6-12 months pre-rituximab, there was a decline in the median relative change of FVC of -2.4% (IQR -7.1 to +0.8). Ten patients had clinically significant PFT progression. In the following 6-12 months post-rituximab, numerical improvement was seen in the median relative change of FVC of +1.2% (IQR -6 to +8.6), median difference +4.2%; p=0.025 (Figure 1A). Similar numerical improvement was seen in the median relative change of DLCO; -4.4% (IQR -11.8 to -3.2) pre-rituximab versus -1.3% (IQR -8.7 to +6.4) post-rituximab, median difference +3.7%; p=0.045 (Figure 1C). PFT progression was halted in 5/10 (50%) of the patients while the remaining 5/10 (50%) continued to progress. Post-rituximab, 7/37 (19%) had improvement in PFT, 25/37 (68%) were stable and 5/37 (13%) had worsening of PFT.

ILDS

Fourteen pairs (pre- and post-rituximab) of HRCT were performed in selected patients with worsening dyspnoea and/or deterioration in lung function. Of these, 1 (7%) had improved (Figure 2), 6 (42%) remained stable and 7 (50%) had worsening of scan imaging appearances post-rituximab. There was no difference in the median pre-rituximab ILDS between patients who had worsening and those who were stable; p=0.26. The inter-rater agreement for the presence or absence of PF (κ=0.63) and HC (κ=0.73) were good, whereas the inter-rater agreement for pure GGO was fair; κ=0.29.

Overall lung response

After rituximab (at the latest time-point with evaluable data for lung assessment), 7/44 (16%) had improved, 23/44 (52%) were stable and 14/44 (32%) ILD progressed. Details of individual lung response are described in the Supplementary Table S1, available at Rheumatology online. Of those whose ILD progressed, 11/14(79%) had pre-existing severe and progressive ILD (lung progression defined as above) with median DLCO of 42% predicted (IQR 41-49) pre-rituximab. 3/14 (21%) whom had stable pre-existing ILD progressed post-rituximab.

Of those with a severe ILD ie: DLCO≤40% recorded at rituximab initiation, stabilisation of ILD post-rituximab were seen in 4/6 (67%) of the patients. The cumulative mortality rate due to ILD progression at 3, 5 and 7 years were 13%, 16% and 16% respectively.

Of the remaining 12 patients with incomplete data for respiratory investigations, 6 (50%) continued on rituximab with no lung exacerbation, 3 (25%) switched therapy due to secondary non-response in terms of RA and 3 (25%) died of non-ILD progression cause (Table 3).

Factors associated with ILD progression

Patients whose lung function deteriorated post-rituximab had a previous history of ILD progression (defined as documented radiographic or PFT progression since diagnosis), radiographic pattern of UIP and lower pre-rituximab DLCO compared to those who were stable or improved (Table 2). The ROC indicated that a pre-rituximab DLCO of 46% predicted which demonstrated 67% sensitivity and 88% specificity in predicting ILD progression after therapy (Figure 3A).

By Kaplan-Meier analysis, radiographic pattern of UIP, a previous history of ILD progression and pre-rituximab DLCO<46% predicted were associated with time-to-ILD progression; p=0.020 (Figure 3B), p=0.001 (Figure 3C) and p=0.001 (Figure 3D) respectively. Smoking and concomitant treatment with csDMARDs were not associated with time-to-ILD progression; p=0.773 (Supplementary Figure S1A) and p=0.260 (Supplementary Figure S1B, available at Rheumatology online, respectively). Details of other clinical risk factors evaluated for lung progression can be found in the Supplementary file, available at Rheumatology online.

There was no significant association between incomplete B-cell depletion and ILD progression in C1; p=0.268. However, a high rate of incomplete depletion was observed in this cohort in

C1; 25/38 (67%). Of those whose ILD progressed, 9/11 of the patients (B-cell data available) had incomplete B-cell depletion post-rituximab in C1. Of 6/9 patients who were re-treated with rituximab, depletion occurred in 2/6.

Factors associated with stabilisation or improvement of ILD

Of those whose ILD improved, 3/7 of the patients had a radiologic pattern of NSIP pre-rituximab, 3/7 UIP and 1 cryptogenic organizing pneumonia (COP). Of those with a radiologic pattern on NSIP pre-rituximab (n=33), patients whose ILD improved or stable post-rituximab had a lower median relative change in DLCO pre-rituximab compared to those who progressed post-rituximab, -3.8% versus -17.5%; p=0.037. Baseline DLCO, ILD duration, concomitant therapies with csDMARDs, corticosteroid and previous treatment with cyclophosphamide were not associated with stabilisation/improvement of ILD post-rituximab in this group of patient; all p>0.10.

Safety

Seventy eight SAEs were recorded in 33 patients: 63 were hospitalisation (median duration 8.5 days) and 3 malignancies (Supplementary Table S2, available at Rheumatology online). Of the 12 deaths, 9 were due to progressive ILD with a median DLCO of 41% predicted pre-rituximab. Other deaths were: 1 lung cancer, 1 colorectal carcinoma and 1 infection post-surgery (Table 3). Median time from last rituximab infusion to death was 11.5 months (range 6-72).

Fifteen serious infections (7.7/100 patient-years) were recorded in 12 patients, mostly due to chest infection. 20% (n=3) and 60% (n=9) of the infections occurred within 3 and 6 months respectively from the last rituximab infusion. 5/12 patients (42%) who had serious infections were also on concomitant therapy with corticosteroid at the cycle when the infection occurred. Details regarding the association of secondary hypogammaglobulinaemia-related to rituximab with serious infection can be found in the Supplementary file and Supplementary Table S2, available at Rheumatology online.

DISCUSSION

This is the largest observational study to date of patients with RA-ILD treated with rituximab. The majority of patients with ILD (as assessed by PFTs, imaging and survival) remained stable or improved after treatment with rituximab over a prolonged follow-up period.

Our data are important to ameliorate reporting bias from case reports or series (5, 6, 15, 16) Data from the British Society of Rheumatology Registry found no increase in overall mortality using TNF-i compared to csDMARDs but a larger proportion of deaths in TNF-i treated patients were attributable to ILD (17). This study might have been affected by: a reporting bias for this event of particular interest; confounding regarding severity of ILD pre-treatment in each treatment group; and channelling away of patients from TNF-i as this was already the established practice during the collection of these data (18). The last could lead to increase reports of adverse events from registry data on the use of rituximab owing to the high morbidity and mortality that are associated with ILD. This present study also was affected by the last issue. However, by reviewing records of every patient who received rituximab in a large cohort to capture every ILD patient with long-term follow-up data in a systematic way, our data helps to avoid reporting bias and the difficulties in interpretation that affect case reports and registry data. The data show that rituximab appears to be generally safe.

It is worth noting that rituximab was given primarily for articular symptoms in this study. Although only 16% of the patients had improvement in ILD after therapy, post-rituximab HRCT was not routinely performed in all patients (if stable), which could reduce the calculated response rate. A high rate of incomplete B-cell depletion as measured using HSFC was observed in C1. Only 1/3 of those whose ILD progressed had complete B-cell depletion when retreated within 12 months. Thus, these might suggest resistance with residual inflammation involving other organs despite response in articular symptoms. Despite the fact that the serial HRCT scans were only undertaken in selected patients with worsening dyspnoea and/or deterioration in PFT, only half of those cases showed radiographic progression. Together with the finding that only 3 patients with stable pre-existing ILD progressed post-rituximab, even without knowledge of HRCT progression in the whole cohort, it is reasonable to infer that clinically significant progression is uncommon with therapy.

About a third of the patients had progression of ILD post-rituximab in this study. This rate was similar to the 34% published by Dawson et al (19) of patients with pre-existing RA-ILD who were treated with csDMARDs over 2 years follow-up period. The demographic, baseline PFT

and definition of lung progression were similar between these two cohorts. However, the present study population was of patients who had failed non-biological DMARDs, with a worse prognosis for both joint and lung disease. In comparison to the use of rituximab in other connective tissue disease (CTD), the rate of ILD progression in this study was also similar to the 15% reported by Keir et al (20). However in the latter, rituximab was given as a rescue therapy for severe and progressive ILD with a median DLCO of 24.5% at rituximab initiation.

With regards to mortality, 9/56 (16%) of the patients died from progressive ILD in this study. The survival rates at 3, 5 and 7 years (87%, 84% and 84% respectively) were similar to the data presented by the British Rheumatoid Interstitial Lung (BRILL) network of patients with RA-ILD treated with rituximab, with survival rates at 3, 5 and 7 years of 92%, 82% and 82% respectively (21). Patients who deteriorated/died in this present study had severe and progressive ILD pre-rituximab, limited reserve and limited treatment options having already failed non-biological DMARDs. Additionally, due to the length of time elapsed from last rituximab infusion before death, the drug was unlikely to be contributory.

We identified three baseline factors that were associated with ILD progression post-rituximab; radiographic pattern of UIP, a previous history of lung progression, and pre-rituximab DLCO<46% predicted. The second concurred with other studies that patients with HRCT findings typical of UIP, have poorer prognosis than individuals with HRCT-detected features indicative of other types of interstitial pneumonia including NSIP (22-24). The last DLCO cut-off in this study is slightly higher than that of criterion referral for lung transplantation (25). These added risks may prompt careful monitoring for lung function when initiating rituximab. Patients with further decline in lung function post-rituximab could be considered for other alternative treatments including cyclophosphamide (26, 27) and lung transplantation (28).

This study has several limitations. First, the PFTs were not undertaken in a standardised manner in all patients. As a result, the efficacy of rituximab in ILD was likely to be underestimated as the 12 patients with missing data for lung investigations (who were not observed to have clinical exacerbation of ILD during therapy) were excluded in the calculated overall lung response analysis. Next, concomitant therapy with csDMARDs were used in more than 60% of the patients, in line with the current licensed indication in RA, thus the effect on lung progression could not be attributed to rituximab alone. Other limitations included variability in rituximab retreatment schedules and difficulty in the interpretation of HRCT due to various patterns seen in rheumatoid lung. Although the inter-rater agreement for GGO was only fair,

the discrepancy was resolved by consensus. Lastly, the lack of a control group made interpretation of the effect of rituximab on the natural course of ILD difficult. In order to account for this, patients were used as their own controls (with pre- and post-treatment lung function trends) and provided convincing evidence of a real treatment effect attributable to rituximab.

Although efficacy signal was demonstrated in some patients with severe ILD pre-rituximab, the fact that others continued to progress despite therapy argued against its therapeutic benefit particularly those who needed treatment the most with respect to ILD. This observation suggest the importance of early diagnosis and treatment. Biomarkers for sub-clinical ILD are emerging (29, 30) and may help stratify patients for early therapy.

To conclude, in the absence of similar data on the effect of other non-B cell depletion therapy on the progression of RA-ILD, our findings offer reassurance that rituximab appears to be an acceptable treatment choice for a group of patients with a non-overlapping RA-ILD and severe arthritis who require a biologic. These data also support a definitive study of rituximab for the management of RA-ILD from both an articular and a respiratory perspective.

Acknowledgements

The authors would like to thank clinicians at the Leeds Biological Monitoring Clinic particularly Maya Buch and Sarah Bingham and to Sue Watts, Respiratory Physiologist at St James' University Hospital, Leeds Teaching Hospitals NHS Trust for their substantial contributions to the acquisition of the data.

Dr Md Yusof is funded as a National Institute for Health Research (NIHR) Doctoral Research Fellow and Dr Vital is funded as an NIHR Clinician scientist.

Conflict of Interest Statement:

Dr Md Yusof is an NIHR Doctoral Research Fellow and has no conflict of interest.

Dr Vital is an NIHR Clinician Scientist. He has received honoraria and research grant support from Roche and GSK.

Dr Dass has received honoraria from Roche and GSK.

Professor Emery has received consultant fees from BMS, Abbott, Pfizer, MSD, Novartis, Roche and UCB. He has received research grants paid to his employer from Abbott, BMS, Pfizer, MSD and Roche.

Miss Kabia, Dr Darby, Dr Lettieri and Dr Beirne have declared no conflict of interest.

Funding: No specific funding was received from any funding bodies in the public, commercial or not-for-profit sectors to carry out the work described in this manuscript.

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Table 1: Baseline characteristics of the 56 rheumatoid arthritis-related interstitial lung disease patients

Age at first RTX infusion, years, median (IQR)	64 (59-72)
Female patient, N (%)	36 (64)
RF positive, N (%)	53/56 (95)
ACPA positive, N (%)	42/51 (82)
Anti-ENA positive[a], N (%)	6/56 (11)
RA Disease duration at first RTX, years, median (IQR)	10 (7-13)
ILD Disease duration at first RTX, years, median (IQR)	5 (3-7)
Smoking status, N (%)	
Never	24 (43)
Ex-smoker	25 (45)
Current	7 (12)
Prior TNF-i treatment, N (%)	16 (29)
Secondary non-response for RA, N (%)	6 (37)
Worsening of ILD, N (%)	10 (63)
Prior CYC therapy for ILD, N (%)	10 (18)
Cumulative dose of CYC, grams, mean (SD)	6.7 (2.5)
No. prior immunosuppressant failure (including TNF-i and CYC but excluding steroid), median (range)	3 (1-9)
Concomitant DMARDs, N (%)	
Methotrexate	28 (78)
Azathioprine	5 (14)
Leflunomide	2 (5)
Mycophenolate Mofetil	1 (3)
DAS-28 at first RTX infusion, mean (SD)	5.64 (1.17)
CRP at first RTX infusion, mean (SD)	30.4 (33.2)
Radiographic pattern of ILD, N (%)	
NSIP	33 (60)
UIP	20 (36)
COP	2 (3)
AIP	1 (1)
FVC (% predicted) at first RTX infusion, median (IQR)	87 (76 – 108)
DLCO (% predicted) at first RTX infusion, median (IQR)	58 (43 – 63)
Expert ILDS at first RTX infusion, median (range)	6 (2-8)

[a]Six patients had concurrent anti-Ro antibody positivity at rituximab initiation. Of these, four had strongly positive ACPA titres and the remaining two (without ACPA positivity) had erosive RA. AIP: Acute interstitial pneumonia; ACPA: Anti-cyclic citrullinated peptide antibody; COP: Cryptogenic organizing pneumonia; CYC: Cyclophosphamide; DAS-28: Disease activity score in 28 joints; DLCO: Carbon monoxide diffusing capacity; DMARDs: Disease modifying anti-rheumatic drugs; ENA: Extractable nuclear antigen; FVC: Forced vital capacity; ILDS: Interstitial lung disease score; NSIP: Non-specific interstitial pneumonia; RTX: Rituximab; TNF-i: Tumour necrosis factor inhibitor; UIP: Usual interstitial pneumonia.

Table 2: Baseline risk factors for lung progression following treatment with rituximab

Characteristics	RA-ILD patients who had lung progression (n=14)	RA-ILD patients with stable/improved lung (n=30)	P-value
Age, years, median (IQR)	70 (61-73)	63 (59-68)	0.302
Male, N (%)	8 (57)	8 (27)	0.091
ILD disease duration, years, median (IQR)	5.5 (3-9)	6.0 (3-7)	0.678
Previous history of lung progression, N (%)	11 (79)	6 (20)	0.001*
Ever smoking, N (%)	8 (57)	15 (50)	0.752
Concomitant DMARDs, N (%)	7 (54)	19 (66)	0.322
Corticosteroid dose, mg, median (IQR)	7.5 (1.9-10)	0 (0-5)	0.054
Radiographic pattern of UIP, N (%)	9 (64)	8 (29)	0.045*
CRP at first rituximab infusion, median (IQR)	24 (12-35)	12 (1-41)	0.348
DLCO at first rituximab infusion, % predicted, median (IQR)	42 (41-49)	59 (54-64)	0.031*

Mann-Whitney U and Fisher's exact tests were used appropriately to test for differences between groups. *$p<0.05$ significant results. DLCO: Carbon monoxide diffusing capacity; RA-ILD: Rheumatoid arthritis-related interstitial lung disease; RTX: Rituximab; UIP: Usual interstitial pneumonia.

Table 3: Causes of deaths in the 12 rheumatoid arthritis-related interstitial lung disease patients

No	Pattern of ILD	FVC Pre-RTX	DLCO Pre-RTX	No of Cycles	Months since last RTX	Cause of death
1	UIP	70	57	4	6	Pneumonia. ILD progression despite CYC and RTX
2	UIP	95	64	3	8	Pneumonia. ILD progression. On home oxygen (LTOT)
3	UIP	N/A	N/A	3	10	ILD progression
4	UIP	79	57	3	11	ILD progression. On LTOT
5	UIP	N/A	N/A	3	12	ILD progression
6	UIP	N/A	41	2	30	ILD progression despite CYC was added. Awaiting lung transplant
7	UIP	72	41	1	36	Pneumonia. ILD progression. On LTOT
8	UIP	N/A	N/A	2	72	Gastrointestinal bleeding secondary to colon cancer
9	NSIP	111	66	1	6	Infection post-neck surgery (atlanto-axial subluxation)
10	NSIP	53	41	1	9	ILD Progression. Awaiting lung transplant
11	NSIP	103	88	2	12	Metastatic lung cancer
12	NSIP	N/A	35	1	15	Pneumonia. ILD progression despite CYC and RTX

DLCO: Carbon monoxide diffusing capacity; FVC: Forced vital capacity; ILD: Interstitial lung disease; LTOT: Long-term oxygen therapy; N/A: Not available; NSIP: Non-specific interstitial pneumonia; RTX: Rituximab; UIP: Usual interstitial pneumonia.

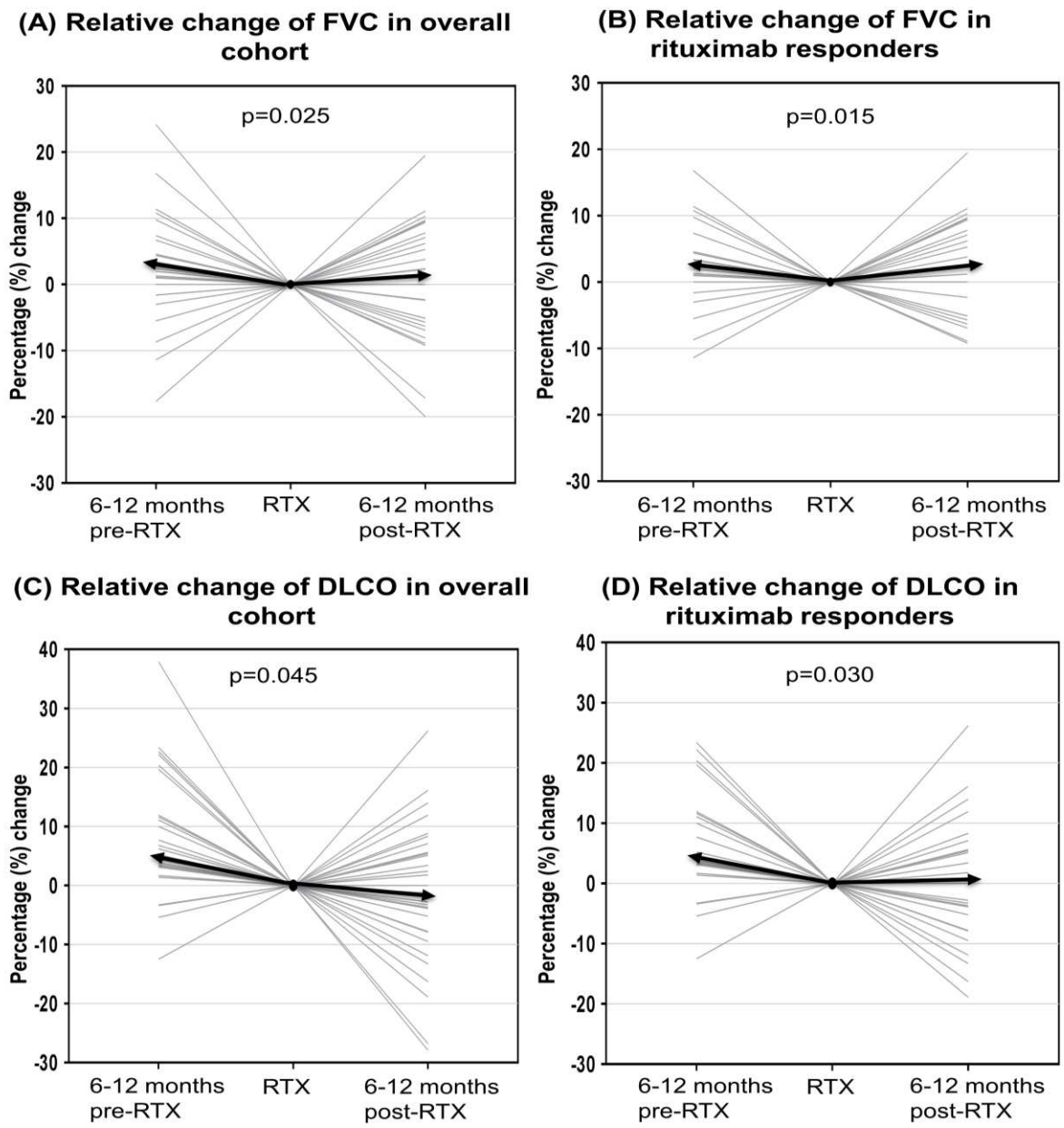


Figure 1: Pre- and post-treatment lung function trends. (A) Overall, the median relative change of FVC was -2.4% (IQR -7.1 to +0.8) pre-rituximab as compared with +1.2% (IQR -6 to +8.6) post-rituximab, representing a difference of +4.2%; p=0.025. (B) For rituximab responders (improvement or stable PFT), the median difference in change of FVC between pre-rituximab versus post-rituximab was +5.1%; p=0.015. (C) Overall, the median change of DLCO was -4.4% (IQR -11.8 to -3.2) pre-rituximab as compared with -1.3% (IQR -8.7 to +6.4) post-rituximab group, representing a difference of +3.7%; p=0.045. (D) For rituximab responders, the median difference in change of DLCO between pre-rituximab versus post-rituximab was +4.4%; p=0.030. Median change for each graph is represented by the solid black arrow. DLCO: Carbon monoxide diffusing capacity, FVC: Forced vital capacity.

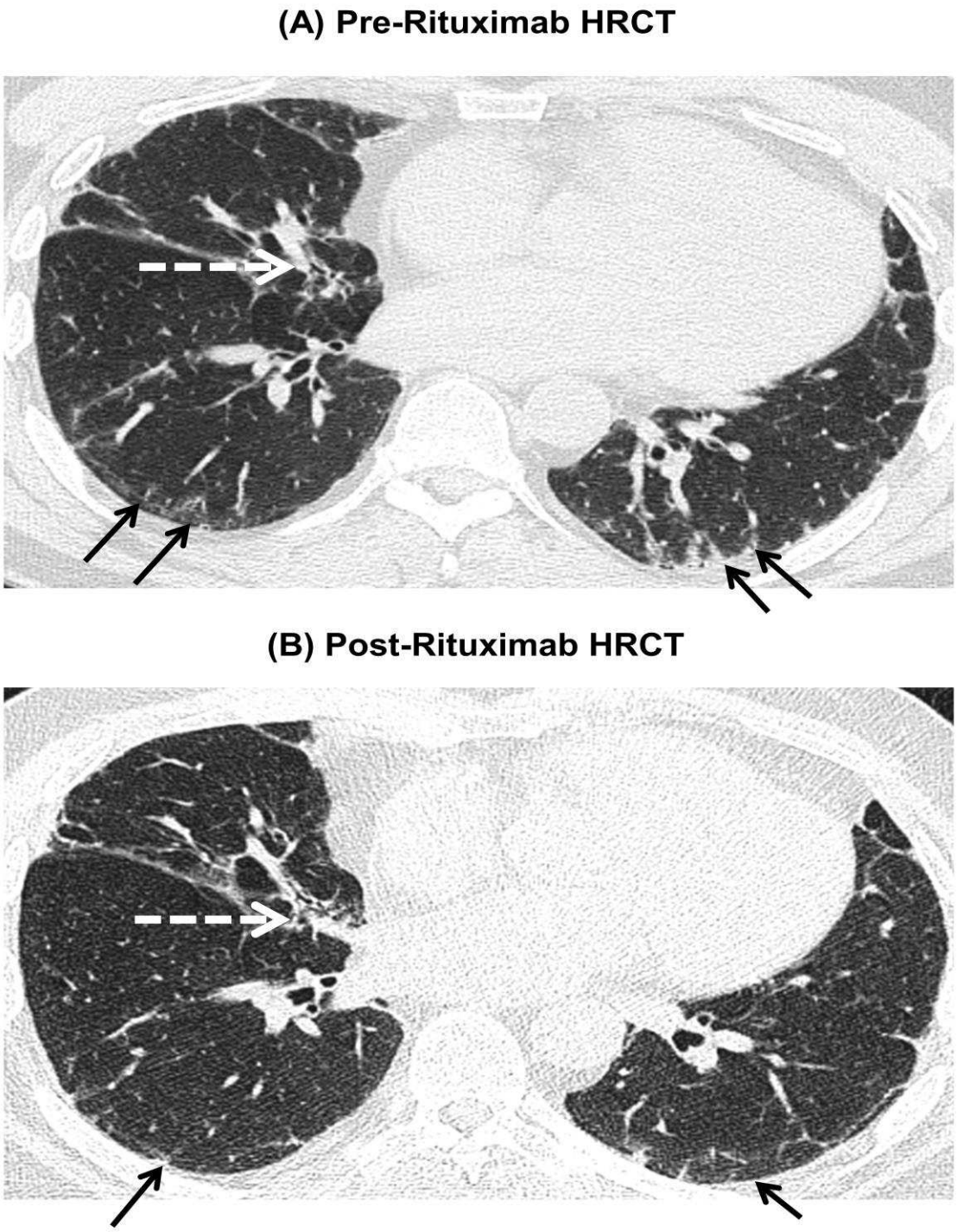


Figure 2: Improvement of high resolution computer tomography 6 months post-rituximab

The black arrow denotes intra- and interlobular thickening while the white arrow denotes traction bronchiectasis. There was an improvement of peripheral fibrosis in B compared to A following treatment with rituximab. HRCT: High resolution computer tomography.

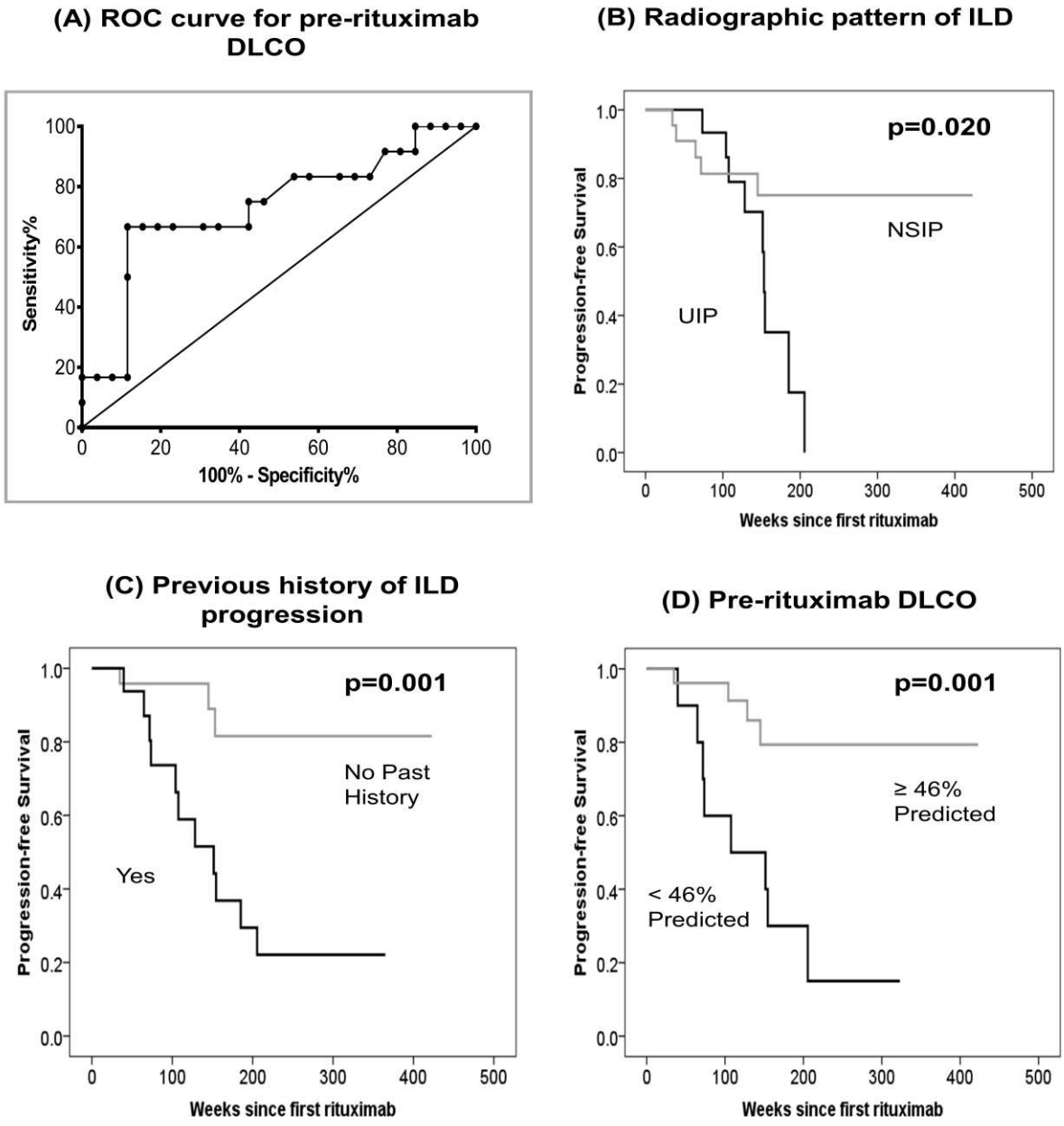


Figure 3: Receiving operator curve and risk factors for lung progression

(A) The best cut-off point for DLCO was 46% predicted, which demonstrated 67% sensitivity and 88% specificity for prediction of ILD progression post-rituximab. Progression-free survival according to (B) radiographic pattern of UIP (C) a previous history of lung progression, and (D) DLCO<46% predicted pre-rituximab; all of which were associated with time-to-ILD progression post-rituximab. DLCO: Carbon monoxide diffusing capacity, ILD: Interstitial lung disease, UIP: Usual interstitial pneumonia

SUPPLEMENTARY DATA

Details methodology for HRCT scans and Immunoglobulin measurement

HRCT scans were acquired (when clinically indicated) in patients with worsening dyspnoea and/or deterioration in lung function using a standardised method at two sites; Leeds General Infirmary Hospital and St James' University Hospital, Leeds. The scans were scored independently by two radiologists; MD – a chest radiologist with over 10 years' experience in reporting ILD; and GL – a general radiologist; both blinded to lung function information and the sequence of scans. ILD Score (ILDS) was used to evaluate the presence and extent of pure ground glass opacification (GGO), pulmonary fibrosis (PF) and honeycombing (HC) in the six lung zones; with a maximum total score possible of 24. The definition of each HRCT variable is as following: pure GGO - increased lung attenuation in the absence of reticular interstitial thickening or architectural distortion; PF - reticular interlobular interstitial thickening, traction bronchiectasis and bronchiolectasis; and HC - clustered air-filled lung cysts with contiguous walls). Each paired scan (pre- and post-treatment) was then rated as 0=worsening, 1=same and 2=improving. Any discrepancy was resolved by consensus.

Total serum immunoglobulin titres were measured by nephelometry at baseline and at 4-6 months after each cycle of rituximab (normal range for IgM: 0.5-2.0 g/L; IgA: 0.8-4.0 g/L and IgG: 6.0-16.0 g/L).

Other important clinical risk factors evaluated for ILD progression

Concomitant csDMARDs was not associated with time-to-ILD progression; p=0.773. Of the 36 patients who were on concomitant csDMARDs at rituximab initiation, data for overall lung response was available for 28 patients. Of these, stabilisation or improvement of ILD occurred in 17/21 (81%) who were on concomitant methotrexate, 2/5 (40%) on azathioprine and the remaining two patients who were each on leflunomide and mycophenolate mofetil. It is

important to note that rituximab was given primarily to treat arthritis and not ILD in this study. Although we can say that concomitant methotrexate appears to be safe, we cannot extrapolate this to those who are treated with rituximab for progressive ILD. Evidence of safety for csDMARDs other than methotrexate is scarce due to small number of patients.

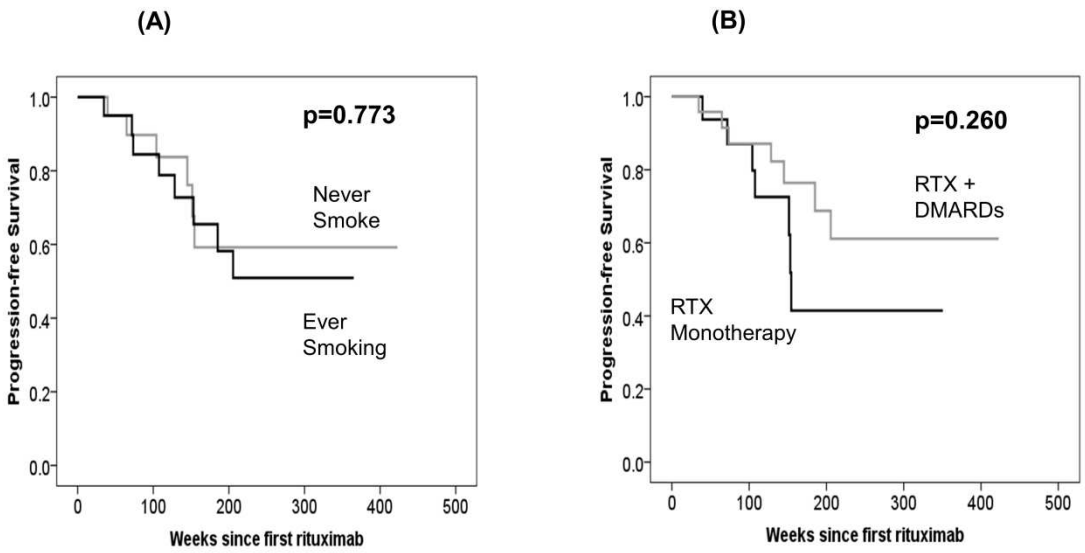
With regards to concomitant corticosteroid use, there was a trend of the non-responders to have higher corticosteroid dose at baseline compared to the responders although this was not statistically significant; p=0.054.

Lastly, as rituximab was approved by the National Institute for Health and Care Excellence (NICE) in the UK in August 2008, we divided the period of recruitment into tertiles; 2004-2008; 2009-2011; and 2012-2015. Of the data with overall lung response available, the rates of responders ie: stabilisation or improvement in ILD for the three categories above were 9/15 (60%), 11/15 (73%) and 12/16 (75%) respectively. Therefore, a period effect in relation to lung response to rituximab is unlikely.

Immunoglobulin and serious infection

In C1, there was a reduction in the mean IgG at 6 months compared to baseline, 11.37 g/L versus 12.97 g/L, mean difference -2.22 g/L (95% CI 3.05 to -1.40); p<0.001. However after 4 cycles of therapy, repeat cycles of rituximab on clinical relapse did not result in significant progressive deterioration in IgG level compared to baseline (p = 0.078) (online supplementary Table S2). Only 2 (4%) patients developed low IgG after rituximab and both had serious infections.

Supplementary Figure S1: Other clinical risk factors evaluated for lung progression



Progression-free survival according to (A) smoking status and (B) concomitant therapy with csDMARDs including methotrexate, azathioprine, leflunomide and mycophenolate mofetil. Both factors were not associated with time-to-ILD progression. csDMARDs: Conventional synthetic disease modifying anti-rheumatic drugs.

Supplementary Table S1: Individual lung response in 44 patients (data available) treated with rituximab

No	Pattern of ILD	Cycle of RTX received	Time Point for PFT/HRCT since first RTX (years)	FVC (Post-RTX)	DLCO (Post-RTX)	ILDS (Post-RTX)	Survival Status (Post-RTX)	Overall Response (Post-RTX)
1	NSIP	1	0.7	S	S	S	A	S
2	NSIP	5	5.3	S	S	S	A	S
3	NSIP	8	6.0	I	S	I	A	I
4	NSIP	1	1.3	S	S	N/A	A	S
5	NSIP	1	2.4	S	S	W	A	W
6	UIP	3	N/A	N/A	N/A	N/A	D	W
7	NSIP	3	2.7	S	W	N/A	A	W
8	UIP	2	2.5	N/A	N/A	S	A	S
9	AIP	7	5.5	S	S	N/A	A	S
10	NSIP	2	1.2	S	S	W	A	W
11	UIP	4	2.4	S	S	W	A	W
12	NSIP	1	0.7	N/A	N/A	S	D	W
13	NSIP	1	0.6	S	S	N/A	A	S
14	UIP	1	2.2	W	S	W	A	W
15	NSIP	2	1.2	S	S	N/A	A	S
16	NSIP	6	4.0	S	S	N/A	A	S
17	NSIP	4	3.8	S	S	N/A	A	S
18	NSIP	3	2.0	S	S	N/A	A	S
19	NSIP	6	4.8	S	S	N/A	A	S
20	UIP	3	3.0	S	S	N/A	A	S
21	UIP	1	6.4	I	S	N/A	A	I
22	UIP	4	2.6	S	S	N/A	A	S
23	NSIP	5	5.0	I	S	N/A	A	I
24	NSIP	4	7.7	S	S	N/A	A	S
25	NSIP	2	1.2	I	S	N/A	A	I
26	NSIP	6	6.4	S	S	N/A	A	S
27	NSIP	6	4.1	I	I	N/A	A	I
28	UIP	3	N/A	N/A	N/A	N/A	D	W
29	UIP	3	N/A	N/A	N/A	N/A	D	W
30	UIP	1	N/A	N/A	N/A	N/A	D	W
31	UIP	2	1.1	S	S	S	A	S
32	UIP	2	1.8	W	S	W	D	W
33	NSIP	1	1.6	S	S	N/A	A	S

34	COP	2	1.3	S	I	N/A	A	I
35	NSIP	1	0.6	S	S	N/A	A	S
36	UIP	2	3.8	I	I	S	A	I
37	UIP	3	2.5	S	S	W	D	W
38	UIP	2	4.3	S	S	N/A	A	S
39	NSIP	1	0.6	S	S	W	D	W
40	UIP	3	N/A	N/A	N/A	N/A	D	W
41	NSIP	5	6.5	S	S	N/A	A	S
42	NSIP	2	5.0	S	S	N/A	A	S
43	NSIP	4	3.2	S	S	N/A	A	S
44	UIP	1	0.6	S	S	N/A	A	S

A: Alive, AIP: Acute interstitial pneumonia, COP: Cryptogenic organizing pneumonia, D: Dead, DLCO: Carbon monoxide diffusing capacity, FVC: Forced vital capacity, HRCT: High resolution computer tomography, ILDS: Interstitial lung disease score, I: Improving, N/A: Data not available, NSIP: Non-specific interstitial pneumonia, PFT: Pulmonary function test, RTX: Rituximab, S: Stable, UIP: Usual interstitial pneumonia, W: Worsening

Supplementary Table S2: Severe adverse events

All severe adverse events	
Number of severe adverse events	78
Patients with severe adverse events, N (%)	33 (59)
All serious infection, number of events	15
Pneumonia	11
Cellulitis	1
Diverticulitis	1
Liver abscess	1
Septic arthritis	1
All other hospitalisation, number of events	48
Orthopaedics surgery (Elective)	14
Lung flare	7
Arthritis flare	6
Gastrointestinal surgery	2
Thromboembolism	2
Acute coronary syndrome	2
Palliative care	2
Seizure	1
Other medical	12
All malignancy, number of events	3
Metastatic lung cancer	1
Colon cancer	1
Metastatic bladder cancer	1
Deaths	12

Supplementary Table S2: IgG levels measured at 6 months of each cycle of rituximab infusion

Time point	Number patients treated	IgG, mean (SD)	p value (versus previous cycle)[a]	Patients with low IgG, N (%)[b]	Patients with new low IgG since previous cycle, N (%)
Baseline	56	12.97 (4.13)	N/A	0/51 (0)	0 (0)
Cycle 1 6 months	56	11.37 (3.59)	p<0.001	2/42 (5)	2/42 (5)
Cycle 2 6 months	41	11.01 (2.94)	0.504	0/26 (0)	0/26 (0)
Cycle 3 6 months	30	10.88 (3.26)	0.526	0/15 (0)	0 (0)
Cycle 4 6 months	21	10.91 (2.50)	0.151	0/10 (0)	0 (0)

Multiple comparisons between IgG levels measured at 6 months of each cycle to baseline IgG showed overall p value of 0.078 using one-way analysis of variance (ANOVA) adjusting for Bonferroni correction. [a]p value was calculated using paired T-test. [b]values show numbers with data available.