Rituximab and Cyclophosphamide in anti-synthetase syndrome (ASyS)-related ILD: an observational retrospective study

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Abstract

Objective: Anti-synthetase syndrome (ASyS)-related interstitial lung disease (ILD) has a poor prognosis. Intravenous cyclophosphamide (CYC) and rituximab (RTX) are the main treatments currently used for moderate to severe ILD. We compare the efficacy of CYC followed by standard immunosuppressive treatment (IST) *vs*. RTX in ASyS-related ILD.

Methods: This observational retrospective study was conducted between 2003 and 2016 in three tertiary care centers. All patients with ASyS-related ILD and treated with CYC or RTX with at least six months of follow-up were included. Pulmonary progression-free survival (PFS) - defined according to the American Thoracic Society guidelines - was assessed at 6 months and 2 years. All severe adverse events were recorded.

Results: Sixty-two patients were included. Thirty-four received 2-12 monthly intravenous CYC pulse, followed by standard IST in 30 cases (88%). RTX-group included 28 patients. Following initial day 1-day 15 infusions, RTX was repeated every 6 months in 26 cases (93%) and 15 patients (54%) received concomitantly another IST. Median steroid dose was similar between both groups. Although RTX and CYC demonstrated similar PFS at 6 months (92% *vs.* 81%, respectively), RTX was superior at 2-years (Hazard Ratio = 0,263 [0,094 - 0,732], p=0.011). Interestingly, lower DL_{CO} at baseline was independently predictive of poor 2-year PFS (0.965 [0.936-0.995], p=0.023). FVC and DLCO improved in both groups without significant difference. Serious adverse events were similar in both groups.

Conclusion: Despite similar PFS at 6 months, RTX was associated with a better 2-year PFS compared to CYC in patients with ASyS-related ILD.

Introduction

Anti-synthetase syndrome (ASyS) is a heterogeneous autoimmune diseases (1) characterized by the presence of inflammatory myopathy, interstitial lung disease (ILD), Raynaud's phenomenon (RP), fever, arthritis, mechanic's hands (2–5), and serum auto-antibodies to aminoacyl-transfer-RNA-synthetases (anti-ARS). Recent work have reported a characteristic myopathological pattern and ASyS is now considered a distinct subset of inflammatory myopathy (6,7,8)

ILD has been reported in over 70% of ASyS patients (1), and is the key prognosis factor in terms of morbidity and mortality (1,9–12). Nonetheless, assessment of ILD severity at diagnosis is currently difficult (13). Appropriate immunosuppressive therapy choice is challenging and based on the results of small retrospective studies (14–19). To date, no prospective trial has validated any IST (20). In Europe, intravenous cyclophosphamide (CYC) has been preferred for the treatment of severe and/or acute-onset ASyS-related ILD. This therapeutic strategy has been largely extrapolated from systemic sclerosis related-ILD treatment (21) and few retrospective studies (14,16). Recently, a retrospective work has reported the efficacy of anti-CD20 therapy using Rituximab (RTX) both on muscle (22) and pulmonary involvement (23–26). CYC and RTX therapy have never been compared with one another, either retrospectively or prospectively.

We therefore performed a multicenter retrospective study comparing CYC and RTX efficacy and tolerance in ASyS-related ILD.

Patients and methods

Patients

This retrospective study was conducted in three university hospitals between 2003 and 2016. Recruitment of the patients was done from the database of the hospital pharmacy: in these centers registration of CYC and RTX are mandatory. Patients over 18 were included if they presented: 1) At least two consecutive positive tests for anti-ARS (ELISA or multiplex immunoassay (Luminex®) for Jo1 and/or line-blot assay (Euroimmun®) testing Jo1-PL7-PL12-EJ-OJ), 2) ASyS-related ILD +/- other clinical

manifestations of ASyS, 3) Absence of previous treatment with CYC or RTX and 4) 6 months of follow-up.

Patients were excluded if they: 1) Received both CYC and RTX at the same time (0-6 months), 2) Received CYC or RTX for extra-pulmonary manifestations, 3) 6 months of follow-up after first administration of RTX or CYC, or 4) Presented any confounding situations at enrollment, such as pulmonary infection or heart failure.

Treatment :

We studied two therapeutics strategies : CYC induction followed by IST vs. RTX induction followed by RTX every 6months.

Ethical considerations

This study was approved by local ethical committee and supervised by the reference centre as CPP IDV VI, June 26th 2012. According to French law, patients were reported anonymously. Consent was necessary to be recorded in the center database.

Data collection

Medical records were retrospectively reviewed to collect clinical, immunological, and histological data as well as detailed medical treatments. Clinical outcomes and adverse events occurring over 2years following either CYC or RTX were collected. Imaging data was collected at inclusion, 6 months (+/-2), 1year and 2years (+/- 2 months). Corticosteroids were regularly evaluate by a physician after 1month, 3month, 6month then every 6months depending on the disease evolution.

Definitions

Onset of the disease was defined as the occurrence of any ASyS manifestation (1-5). Anti-ARS-related-ILD was defined as: 1) ILD according to international consensus (27–32) on CT-scan features (review by a first experienced radiologist at the time where CTs was performed (standard of care), and by our experienced radiologist (MLC) who reviewed all of them blindly and retrospectively) and abnormal pulmonary function tests (PFTs) with forced vital capacity (FVC) <70% and/or corrected diffusing lung

capacity for carbon monoxide (cDLCO) <70%**.** and 2) Its association with two positive anti-ARS testing, and 3) The absence of alternative diagnosis (including infectious pneumonia or heart failure…). New York Heart Association (NYHA) Functional classification (33) used to categorize patient-reported function.

American Thoracic Society /European Respiratory Society classification (27,32,34) was used to identify ILD patterns: nonspecific interstitial pneumonia (NSIP), organized pneumonia (OP), usual interstitial pneumonia (UIP) or association of both NSIP and OP.

Like some prospective pulmonary trials (35), we used a composite evaluation to determine ILD course. Improvement of ILD was defined as improvement of at least two of the following features: 1) Clinical features: improvement in NHYA class ; 2) PFT evaluation relative FVC improvement of ≥10% and/or cDLCO improvement of ≥15%(30–32); 3) improvement of ILD extension in the lung parenchyma on CT-scan (36). Conversely, ILD worsening was defined as worsening of at least one of the following features: 1) Clinical features: worsering in NHYA class ; 2) PFT evaluation: Relative FVC decrease of ≥10% and/or cDLCO decrease of ≥15%; 3) CT-scan worsening of ILD extension score. Patients not meeting improvement or worsening definition were considered as stable.

Treatment-related severe adverse events were defined as all treatment-related complications leading to patient's hospitalization or treatment's discontinuation.

Endpoints

Our primary endpoint was the pulmonary progression-free survival, which was defined by the absence of worsening of pulmonary involvement during the follow-up period and the absence of treatment-related severe adverse event.

Statistical analysis

Each group was analyzed using descriptive statistics, based on median, minimum and maximum. CYC and RTX groups were compared using bivariate statistical analyses (non-parametric Mann-Whitney and paired Wilcoxon tests for continuous variables and Fisher's exact tests for categorical variables).

Kaplan-Meier curves were used to describe pulmonary progression-free survival in CYC and RTX groups. Bivariate and multivariate Cox models were used to compare CYC to RTX pulmonary progression-free survival. Only a two-sided p-value <0.05 was considered significant. Analyses were performed using R statistical software (v3.3.1, R-Foundation for Statistical Computing, Austria).

The choice of adjustments was done a priori, from the variables that were most probably confounders, i.e. associated to the prescription of either treatment and associated to the outcome.

Results

Patient selection

Amongst more than 1200 inflammatory myopathies in the database, we identified 214 patients with ASyS. 85 of them (40%) had been treated with CYC or RTX for. Twenty patients were excluded because CYC or RTX had been given for extra-pulmonary manifestations. Three others were also excluded due to simultaneous CYC and RTX treatment. We thus included 62 patients, 34 in the CYC group and 28 in the RTX group, respectively. Patients characteristics are described in Table 1. Year of enrollment was comparable in both groups (p=0,3).

Cyclophosphamide group

At baseline, median MMT8 score was 77 (range 53-80). PFTs showed a median cDLCO of 32% (range 15-65) and FVC of 53% (range 29-121) (Table 2). CT-scan patterns were consistent with NSIP (n=19, 56%), UIP (n=5, 14%), OP (n=2, 6%) and NSIP/OP (n=8, 24%).

CYC was administrated in all patients at 750mg/m2/month intravenously, in association with high-dose steroids. The median number of monthly CYC infusion was 6 [2-12]. Following this “induction” therapy period, CYC was switched to another IST in 30 (88%) patients (azathioprine n=14, mycophenolate mofetil n=12, methotrexate n=2 and ciclosporine/tacrolimus n=2) while steroids were progressively tapered. Monthly intravenous immunoglobulins (IVIg) were also initially administered in 7 patients due to severe muscle involvement.

At six months PFTs showed a significantly increased median FVC and median cDLCO of 53% to 62% (p=0.01) and of 31.5% to 35% (p=0.01), respectively. Over this period, median MMT8 score improved to 80 [72-80] (p=0.006), and median CK level decreased dramatically to 100 IU/L [24-1,860] (p<0.0001).

After six months of treatment, besides this global PFTs improvement, 29/34 patients (85%) achieved the primary endpoint, *i.e* pulmonary progression-free survival (Table 3). Indeed, four patients needed to discontinue CYC before 6months of treatment due to ILD worsening according to our criteria: two patients worsened on PFTs, two on CT-scan, and two on NYHA dyspnea class. One additional patient developed a severe adverse event attributed to CYC (thrombotic microangiopathy).

When considering PFTs at two years (Figure 1), FVC and cDLCO gradually improved during the follow-up. Median FVC increased from 53% at baseline to 80%, corresponding to a non-significant increase of +5% ([-13;+27], p=0.15). Similarly, median cDLCO non significantly increased from 32% at baseline to 55% after 2 years, corresponding to a median increase of +3% after 2 years ([-4;+20], p=0.31). When considering all follow-up data, 8 patients had CT-scan improvement, 14 were stable, 8 had worsened (mean of follow-up = 18,4 month). Of note, 4 patients didn't have a CT-scan at follow-up or had a severe adverse event that made CT-scans uninterpretable. NYHA class decreased from a median of 3 at baseline to 2 at 12 month (p=0.1) and at 2 years (p=0.03).

According to our definitions 8 patients (24%) remained event-free at 2 years. Indeed, 16 patients had ILD worsening (47%): six regarding %CV and/or %DCLO, eight on the basis of ILD extension on CT-scan and three according to NYHA class. In addition, 4 patients were censored (1 lost to follow-up and 3 patients reaching the end of study in 2017) and 6 patients presented a CYC-related severe adverse event (18%) including one early thrombotic microangiopathy, infectious pneumonia (leading to death in one case) and one aseptic meningitis.

Of note, two extra-pulmonary relapses (muscle and joints) occurred during the 2-year follow-up period.

Rituximab group

At baseline, median MMT8 score was 78 (range 59-80). PFTs disclosed a median cDLCO of 45% (range 17-88) and FVC of 64% (range 21-104) (Table 2). CT-scan patterns were consistent with NSIP (n=22, 78%), OP (n=1, 4%) and NSIP/OP (n=5 patients, 18%).

Two infusions of 1g of RTX D1-D15 or a dose of 375mg/m^2 once weekly for four weeks were administered. High-dose steroids were associated in 27/28 patients (96%). All patients had maintenance 1g-RTX infusions every six months. Median number of maintenance RTX infusions was 3 [2-10]. Fifteen patients (54%) also received another IST concomitantly: azathioprine n=6, mycophenolate mofetil n=5, methotrexate n=3, tacrolimus n=1. Monthly IVIg were also administered initially in 2 patients (8%). Yet, 13 patients (46%) were treated with RTX and steroids only.

Median FV -but not median cDLCO- increased significantly at 6 months from 64% to 74% (p=0.002) and from 45% to 46% (p=0.1), respectively. Over this period, median MMT8 score improved up to 80 [65-80] (p=0.015) and median CK level decreased to 128 IU/L [33-5,686] (p=0.01). After six months of treatment, 25 of 28 patients (89%) achieved primary endpoint (Table 3). ILD worsening was observed in two patients on CT-scan only. One patient developed a severe adverse event (acute respiratory distress syndrome).

At two years FVC and cDLCO improved during follow-up period (Figure 1). Median FVC increased significantly from 64% at baseline to 92% at 12 months and 90% after 2 years of follow-up, corresponding to a median increase of +7% at 12 months (p=0.0005) and +7% after 2 years (p=0.008). Although non-significant, median cDLCO increased from 45% at baseline to 48% at 12 months and 60% after 2 years of follow-up(median increase of +1% at 12 months (p=0.4) and +5% after 2 years (p=0.1)). Eleven patients had CT-scan improvement, 12 were stable, and 1 had worsening (mean of follow up = 17,5 months). Four patients couldn't be analyzed due to the lack of CT-scan control or severe adverse events that makes CT-scans uninterpretable. When considering dyspnea, NYHA decreased from a median of 3 at baseline to 1.5 at 12 months (p=0.001) and 1 after 2 years of follow up (p=0.003).

Thus, at 2 years, 15 patients achieved the primary endpoint (event-free survival = 54%), whereas 3 patients had ILD worsening (11%): according to our definitions %CV and/or %cDCLO worsened in one

case, chest CT-scan in two cases but no patients improved their NYHA class. In addition, 8 were censored (2 lost to follow-up and 6 reaching the end of study) and 2 presented a severe adverse event (7%) attributed to RTX, including an acute respiratory distress syndrome and one infectious pneumonia leading to patient's death.

Of note, four extra-pulmonary relapses occurred during the follow-up period (muscle n=2, joints n=2).

Comparison of Rituximab versus cyclophosphamide

To further compare CYC and RTX efficacy and tolerance, we performed bivariate analyses demonstrating few differences between both groups at baseline (Table 1 and 2). i) CYC-group seemed to display worse PFTs than RTX group at inclusion. Indeed, median FVC and cDLCO were significantly lower in CYC-group compared to RTX-group (53% *vs*. 64% (p=0.04) and 32% *vs*. 45% (p=0.01), respectively) (figure 1); ii) RTX-group seemed to have more refractory disease compared to CYC-group as reflected by the number of previous lines of immunosuppressive treatment 2.32 ± 1.45 *vs*. 1.35 ± 1.39 (p=0.004), respectively. Of note, there were no other significant differences among the groups, including clinical, biological and radiological features (Table1 and 2).

Maintenance therapy was strictly different between groups. In the CYC group, the maintenance therapy was mainly Mycophenolate Mofetil and Azathioprine while, in the rituximab group, all patients had rituximab as maintenance therapy. Therefore, groups are not comparable for the maintenance therapy and no adjustment can be made. The comparison is mainly about Ritux followed by Ritux vs CYC followed by Mycophenolate pooled with CYC followed by Azathioprine. The unadjusted difference of PFS between CYC+Mycophenolate vs CYC+Azathioprine adjusted on the treatment line was not significant (HR=0.89, 95% CI: 0.31 to 2.59, p=0.84) but the actual statistical precision was very low, with only 8 events in the CYC+Azathioprine group and 6 events in the Mycophenolate group.

Steroid tapering did not differ between groups as doses were similar between groups at six month (10mg [8;17,5] vs. 10mg [8;15], p = 0.59) and one year (5mg [0-6] vs.5mg [0-8], p = 0.79).Bivariate Cox analyses demonstrated a better pulmonary progression-free survival in RTX-group compared to CYC-group after two years of follow-up (unadjusted Hazard Ratio (HR): 0.223 [95% CI: 0.084-0.59],

p=0.003). A multivariate cox model adjusted for cDLCO and the previous lines of treatment was subsequently performed to overcome baseline differences observed between both groups. At two years follow-up, RTX strategy appears to be a protective factor (adjusted HR (aHR): 0.263 [95% CI: 0.094-0.723], p=0.011). After additional adjustment for the date of treatment initiation, the hazard ratio was estimated at 0.248 (95% CI: 0.089 to 0.691, p=0.008). Furthermore, baseline cDLCO was significantly associated with pulmonary progression-free survival at two years (aHR: 0.964 [95% CI: 0.935-0.994], p=0.019). Previous immunosuppressive lines had a non-significant association with pulmonary progression-free survival at two years (aHR: 1.226 [95% CI: 0.553-2.718], p=0.62) (Table 4).

In order to take into account the impact of losses to follow-up, we performed a maximum bias analysis, assuming that losses to follow-up in the CYC group are all event-free at 2 years, while losses to follow-up in the Rituximab group have an event at the date of loss to follow-up. In the model adjusted on DLCO and treatment line, the effect of Rituximab is still significant (HR=0.369, 95% CI: 0.148 to 0.917, p=0.032).

Proportional hazards assumption might not met according to a proportional hazards χ^2test on Schoenfield residuals (p=0.09). Yet, as there was no obvious inversion of hazards, Cox models were kept. Kaplan Meier survival analyses confirmed these results (Figure 2).

Discussion

ILD is the key prognosis factor in ASyS (1,9,11,12,37). In this study, we describe the largest cohort of patients with ASyS-related ILD treated with CYC or RTX. Our cohort share similar characteristics than other reported cohorts (7,9). However, due to the retrospective nature of our work, recruitment and evaluation bias may have occurred. Of note the medication dosages were unusually and fairly uniform - this is an usually bias in cohort treatment studies - and a strong quality indicator of this study despite being observational. Although patients displayed similar clinical and immunological features in both groups at baseline, we acknowledge that patients in the CYC-group presented a more severe ILD, with lower cDLCO and FVC compared to the RTX-group. This could reflect the physician's preference to use

CYC in severe and/or rapidly progressive ILD. Moreover, RTX therapy has shown its efficacy only recently in ASyS, and is mostly given as second-line treatment. The lack of standardized treatment algorithms also could impact on therapeutic efficacy: yet, patients in the RTX-group presented a more refractory disease course than patients from the CYC-group. Nonetheless, despite these slight differences, we observed a similar evolution of PFTs in both groups after 2 years, when considering FVC and cDLCO as quantitative variables.

Moreover, PFTs which are crucial criteria in IPF (32), could be influenced by respiratory muscle involvement: improvement of muscle involvement could lead to increased FVC, while ILD may worsen. Similarly, analyzing CT-scan could be distorted in case of adverse events, such as pneumonia or heart failure, regarding both the fibrosing and the extension scores of the ILD. Evaluation based solely on PFTs or CT-scan features may thus lead to misinterpretation. Finally, NYHA class is a categorical classification score that is not sensitive enough to change over time to be the sole parameter for describing patients' outcome and do not only evaluate dyspnea but also relates to symptoms including fatigue, muscle weakness or other cofounding parameters. One of our limitations were the absence of a validated dyspnea scale. They were not performed routinely and were not available for our patients. A control prospective study should include it.

cDLCO, which is the PFT-parameter less likely to influenced by muscle involvement, was a good predictive factor in our multivariate analyses: the higher the cDLCO is at inclusion, the better is the 2-year pulmonary free survival. We however did not show any significant variation between the two groups on the basis of PFT parameters. Thus, combining different parameters in a global and composite index, like pulmonary progression-free survival, seemed to be the more relevant choice.

Few studies have specifically evaluated the efficacy of CYC in patients with inflammatory myopathy related-ILD. In these small series (14–19), population and assessment criteria are often heterogeneous. Marie *et al.* (16) showed an improvement of ILD in 16 of 25 patients (64%) and Yamasaki *et al.* (14) have reported an improvement in 13 of 17 patients (75%). We observed herein similar results with a 6-month pulmonary progression-free survival of 82% in the CYC-group, persisting in only 24% after two

years (including censored patients). Six severe adverse events occurred in this group over the study period, most of them (5/6) occurring during the CYC therapy period (at least first six months) rather than during later use of another IST. These results suggested that the "CYC followed by standard IST strategy" allows good short-term results despite low early tolerance.

As a whole, RTX has shown its efficacy in inflammatory myopathies, with over 78% of responses (38). Until now however, RTX use in ARS-related ILD is still rather rare. Two recent retrospective studies suggested however the efficacy of RTX on in ARS-related ILD in terms of both PFTs and CT-scan improvement (24,39). Nevertheless, a 25% mortality rate was reported (24)**.** Mortality being mainly due to severe infections, this suggest to be cautious in patients sometimes treated with previous lines of immunosuppressive drugs. In a prospective study from our group (23), we reported an improvement of the FVC value in 50% of patients with refractory ASyS-related ILD. In the current study, we confirmed the efficacy of RTX as an induction therapy with a 92% of pulmonary progression-free survival at six months. Moreover, at 2 years, 15 patients (54%) were still free of pulmonary progression (including the patients lost to follow-up). Only two patients experienced a severe adverse event in this group, which is fewer than reported in other cohorts (23). Our result demonstrated that RTX has a good efficacy profile and is well tolerated, despite being used in a subset of refractory patients.

To the best of our knowledge, no study had compared the long-term RTX and CYC strategies efficacy and tolerance in ARS-related ILD yet. Both free-pulmonary survivals - 93% and 82%, respectively - were comparable during the first six months of treatment. Importantly and despite similar improvement of PFTs, the CYC and RTX survival curves dramatically and significantly separated after 6 months of treatment. In light of the multivariate analyses, RTX treatment appears to be a clear protective factor regarding ILD progression. When comparing pulmonary progression-free survival using bi- and multivariate Cox model, we were able to show a better 2-year pulmonary progression-free survival in the RTX group as compared to the CYC group. We noticed that this difference was observed from the time of IST introduction, following the CYC. Our data thus confirmed the interest of RTX in ASyS-related ILD and identified two distinct phases in the treatment of patients with ARS-related ILD. First,

the “induction” phase was comparable between RTX and CYC treatment. Second, the “maintenance” phase (after six months of follow-up) was clearly in favor of RTX compared to CYC followed by IST. Of note, there was no difference between patients with low dose of steroids plus RTX alone (13/28) *vs.* RTX associated with another IST (15/28). This difference was persistent in 15 patients for which follow-up was > 2 years (data not shown). Possible explanations might be a lower efficacy of IST compared to repeated RTX infusions which has not been shown fir any standard IST to date (16–18) or a lower compliance to standard IST as compared to RTX infusions which is given intravenously (40).

Considering two distinct phases, our data clearly support both the use of CYC or RTX as induction therapy, but clearly showed the superiority of RTX as a maintenance therapy. Prospective trials are needed to confirm these results and to find best new treatment strategies.

Conclusion

RTX and CYC demonstrated similar efficacy as induction therapy at 6 months in refractory ASyS-related ILD. Promisingly, RTX appeared to be more effective than the other IST as maintenance therapy (after 6 months).

References

1. Hervier B, Devilliers H, Stanciu R, Meyer A, Uzunhan Y, Masseau A, et al. Hierarchical cluster and survival analyses of antisynthetase syndrome: phenotype and outcome are correlated with anti-tRNA synthetase antibody specificity. Autoimmun Rev. 2012;12:210–7.

2. Aggarwal R, Cassidy E, Fertig N, Koontz DC, Lucas M, Ascherman DP, et al. Patients with non-Jo-1 anti-tRNA-synthetase autoantibodies have worse survival than Jo-1 positive patients. Ann Rheum Dis. 2014;73:227–32.

3. Marguerie C, Bunn CC, Beynon HL, Bernstein RM, Hughes JM, So AK, et al. Polymyositis, pulmonary fibrosis and autoantibodies to aminoacyl-tRNA synthetase enzymes. Q J Med. 1990;77:1019–38.

4. Hervier B, Meyer A, Dieval C, Uzunhan Y, Devilliers H, Launay D, et al. Pulmonary hypertension in antisynthetase syndrome: prevalence, aetiology and survival. Eur Respir J. 2013;42:1271–82.

5. Dieval C, Deligny C, Meyer A, Cluzel P, Champtiaux N, Lefevre G, et al. Myocarditis in Patients With Antisynthetase Syndrome: Prevalence, Presentation, and Outcomes. Medicine. 2015;94:e798.

6. Mescam-Mancini L, Allenbach Y, Hervier B, Devilliers H, Mariampillay K, Dubourg O, et al. Anti-Jo-1 antibody-positive patients show a characteristic necrotizing perifascicular myositis. Brain J Neurol. 2015;138:2485–92.

7. Allenbach Y, Benveniste O, Goebel H-H, Stenzel W. Integrated classification of inflammatory myopathies. Neuropathol Appl Neurobiol. 2017;43:62–81.

8. Mariampillai K, Granger B, Amelin D, Guiguet M, Hachulla E, Maurier F, et al. Development of a New Classification System for Idiopathic Inflammatory Myopathies Based on Clinical Manifestations and Myositis-Specific Autoantibodies. JAMA Neurol. 2018;10.2598

9. Lega J-C, Fabien N, Reynaud Q, Durieu I, Durupt S, Dutertre M, et al. The clinical phenotype associated with myositis-specific and associated autoantibodies: a meta-analysis revisiting the so-called antisynthetase syndrome. Autoimmun Rev. 2014;13:883–91.

10. Cottin V, Thivolet-Béjui F, Reynaud-Gaubert M, Cadranel J, Delaval P, Ternamian PJ, et al. Interstitial lung disease in amyopathic dermatomyositis, dermatomyositis and polymyositis. Eur Respir J. 2003;22:245–50.

11. Johnson C, Pinal-Fernandez I, Parikh R, Paik J, Albayda J, Mammen AL, et al. Assessment of Mortality in Autoimmune Myositis With and Without Associated Interstitial Lung Disease. Lung. 2016;194:733–7.

12. Bartoloni E, Gonzalez-Gay MA, Scirè C, Castaneda S, Gerli R, Lopez-Longo FJ, et al. Clinical follow-up predictors of disease pattern change in anti-Jo1 positive anti-synthetase

syndrome: Results from a multicenter, international and retrospective study. Autoimmun Rev. 2017;16:253–7.

13. Fathi M, Vikgren J, Boijsen M, Tylen U, Jorfeldt L, Tornling G, et al. Interstitial lung disease in polymyositis and dermatomyositis: longitudinal evaluation by pulmonary function and radiology. Arthritis Rheum. 2008;59:677–85.

14. Yamasaki Y, Yamada H, Yamasaki M, Ohkubo M, Azuma K, Matsuoka S, et al. Intravenous cyclophosphamide therapy for progressive interstitial pneumonia in patients with polymyositis/dermatomyositis. Rheumatology. 2007;46:124–30.

15. Cavagna L, Caporali R, Abdì-Alì L, Dore R, Meloni F, Montecucco C. Cyclosporine in anti-Jo1-positive patients with corticosteroid-refractory interstitial lung disease. J Rheumatol. 2013;40:484–92.

16. Marie I, Josse S, Hatron PY, Dominique S, Hachulla E, Janvresse A, et al. Interstitial lung disease in anti-Jo-1 patients with antisynthetase syndrome. Arthritis Care Res. 2013;65:800–8.

17. Mira-Avendano IC, Parambil JG, Yadav R, Arrossi V, Xu M, Chapman JT, et al. A retrospective review of clinical features and treatment outcomes in steroid-resistant interstitial lung disease from polymyositis/dermatomyositis. Respir Med. 2013;107:890–6.

18. Takada K, Nagasaka K, Miyasaka N. Polymyositis/dermatomyositis and interstitial lung disease: a new therapeutic approach with T-cell-specific immunosuppressants. Autoimmunity. 2005;38:383–92.

19. Hervier B, Wallaert B, Hachulla E, Adoue D, Lauque D, Audrain M, et al. Clinical manifestations of anti-synthetase syndrome positive for anti-alanyl-tRNA synthetase (anti-PL12) antibodies: a retrospective study of 17 cases. Rheumatology. 2010;49:972–6.

20. Morisset J, Johnson C, Rich E, Collard HR, Lee JS. Management of Myositis-Related Interstitial Lung Disease. Chest. 2016;150:1118–28.

21. Wallace B, Vummidi D, Khanna D. Management of connective tissue diseases associated interstitial lung disease: a review of the published literature. Curr Opin Rheumatol. 2016;28:236–45.

22. Oddis CV, Reed AM, Aggarwal R, Rider LG, Ascherman DP, Levesque MC, et al. Rituximab in the treatment of refractory adult and juvenile dermatomyositis and adult polymyositis: a randomized, placebo-phase trial. Arthritis Rheum. 2013;65:314–24.

23. Allenbach Y, Guiguet M, Rigolet A, Marie I, Hachulla E, Drouot L, et al. Efficacy of Rituximab in Refractory Inflammatory Myopathies Associated with Anti- Synthetase Auto-Antibodies: An Open-Label, Phase II Trial. PloS One. 2015;10:e0133702.

24. Andersson H, Sem M, Lund MB, Aaløkken TM, Günther A, Walle-Hansen R, et al. Long-term experience with rituximab in anti-synthetase syndrome-related interstitial lung disease. Rheumatology. 2015;54:1420–8.

25. Dasa O, Ruzieh M, Oraibi O. Successful Treatment of Life-Threatening Interstitial Lung Disease Secondary to Antisynthetase Syndrome Using Rituximab: A Case Report and Review of the Literature. Am J Ther. 2016;23:639-645.

26. Sharp C, McCabe M, Dodds N, Edey A, Mayers L, Adamali H, et al. Rituximab in autoimmune connective tissue disease-associated interstitial lung disease. Rheumatology. 2016;55:1318–24.

27. Sverzellati N, Lynch DA, Hansell DM, Johkoh T, King TE, Travis WD. American Thoracic Society-European Respiratory Society Classification of the Idiopathic Interstitial Pneumonias: Advances in Knowledge since 2002. Radiographics. 2015;35:1849–71.

28. Grenier P, Chevret S, Beigelman C, Brauner MW, Chastang C, Valeyre D. Chronic diffuse infiltrative lung disease: determination of the diagnostic value of clinical data, chest radiography, and CT and Bayesian analysis. Radiology. 1994;191:383–90.

29. Lynch DA, Travis WD, Müller NL, Galvin JR, Hansell DM, Grenier PA, et al. Idiopathic interstitial pneumonias: CT features. Radiology. 2005;236:10–21.

30. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med. 2000;161:646–64.

31. Saketkoo LA, Mittoo S, Huscher D, Khanna D, Dellaripa PF, Distler O, et al. Connective tissue disease related interstitial lung diseases and idiopathic pulmonary fibrosis: provisional core sets of domains and instruments for use in clinical trials. Thorax. 2014;69:428–36.

32. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med. 2011;183:788–824.

33. Dolgin M, Association NYH, Fox AC, Gorlin R, Levin RI, New York Heart Association. Nomenclature and criteria for diagnosis of diseases of the heart and great vessels. 9th Ed Boston MA Lippincott Williams Wilkins. 1994;

34. Debray M-P, Borie R, Revel M-P, Naccache J-M, Khalil A, Toper C, et al. Interstitial lung disease in anti-synthetase syndrome: initial and follow-up CT findings. Eur J Radiol. 2015;84:516–23.

35. Pulido T, Adzerikho I, Channick RN, Delcroix M, Galiè N, Ghofrani H-A, et al. Macitentan and morbidity and mortality in pulmonary arterial hypertension. N Engl J Med. 2013;369:809–18.

36. MacDonald SL, Rubens MB, Hansell DM, Copley SJ, Desai SR, du Bois RM, et al. Nonspecific interstitial pneumonia and usual interstitial pneumonia: comparative appearances at and diagnostic accuracy of thin-section CT. Radiology. 2001;221:600–5.

37. Pinal-Fernandez I, Casal-Dominguez M, Huapaya JA, Albayda J, Paik JJ, Johnson C, et al. A longitudinal cohort study of the anti-synthetase syndrome: increased severity of interstitial lung disease in black patients and patients with anti-PL7 and anti-PL12 autoantibodies. Rheumatology. 2017;56:999–1007.

38. Fasano S, Gordon P, Hajji R, Loyo E, Isenberg DA. Rituximab in the treatment of inflammatory myopathies: a review. Rheumatology. 2017;56:26–36.

39. Doyle TJ, Dhillon N, Madan R, Cabral F, Fletcher EA, Koontz DC, et al. Rituximab in the Treatment of Interstitial Lung Disease Associated with Antisynthetase Syndrome: A Multicenter Retrospective Case Review. J Rheumatol. 2018;45:841–50.

40. Lamouroux A, Magnan A, Vervloet D. Compliance, therapeutic observance and therapeutic adherence: what do we speak about? Rev Mal Respir. 2005;22:31–4.

Table 1: Patients characteristics at baseline

	All cohort (n=62) Median [Q1-Q3] or %	Rituximab (n=28) Median [Q1-Q3] or %	Cyclophosphamide (n=34) Median [Q1-Q3] or %	*p*-value
Demographics				
Age	54 [45-63]	54 [44-63]	54 [45-60]	0.85
Sex (M/F)	14/48	7/21	7/27	0.77
Disease duration before RTX or CYC (months)	38.5 [10.7-71.3]	46.7 [13.7-80.1]	30.2 [2-53.3]	0.12
Previous lines of treatment	2 [1-3]	2 [1-3]	1 [0-2]	**0.004**
Previous treatments		CTC (96%); AZA (57%); IGIV (36%); MTX (39%); MMF (46%); CSP (7%); aTNF (7%); CYC (43%)	CTC (100%); AZA (32%); IGIV (9%); MTX (24%); MMF (24%); SSZ (6%); aTNF (3%); RTX (3%)	
Clinical features (%)				
Myalgia	82	86	79	0.68
Muscle weakness	71	75	68	0.63
Arthralgia/arthritis	76	86	68	0.23
Cutaneous involvement	55	57	53	0.78
Heart involvement	10	7	12	0.76
Fever	61	64	59	0.72
Cought	87	82	91	0.55
MMT8	78 [71-80]	78 [59-80]	77 [53-80]	0.43
Immunological features (%)				
Jo1	69	82	59	0.12
PL7	18	14	21	0.59
PL12	8	4	12	0.68
Ro52	53	54	53	0.97
Laboratory features				
Hb	12.4 [11.2-13.3]	12 [11-12.8]	12.6 [11.6-13.3]	0.45
CK	722 [188-1683]	735 [210-1491]	766 [340-2032]	0.85
Albumin (n=31)	34 [31-38]	35 [33-38]	33 [31-36]	0.49
CRP	13 [3-35]	11 [0-30]	13 [8-36]	0.19

Q1-Q3: quartile1-quartile3; PHT: Pulmonary hypertension; Hb: hemoglobin; CK: Creatine kinase; CRP: C-reactive protein; IVIG: intravenous immunoglobulin; AZA: Azathioprine; MTX: Methotrexate; MMF: mycophenolate mophetil; CSP: cyclosporine; TACRO: tacrolimus ; aTNF: anti-TNFalfa; SSZ: sulfasalazin.

Table 2: Baseline ILD features

	All cohort (n=62) Median [Q1-Q3]	Rituximab (n=28) Median [Q1-Q3]	Cyclophosphamide (n=34) Median [Q1-Q3]	*p*-value
PFTs				
FVC (% predicted value)	63 [45-75]	64 [54-91]	53 [43-64]	**0.036**
FEV1 (% predicted value)	62 [46-69]	63 [51-72]	56 [44-64]	0.18
TLC (% predicted value)	63 [47-78]	68 [52-82]	55 [44-68]	0.23
cDLCO (% predicted value)	39 [24-52]	45[31-57]	32[24-39]	**0.01**
Dyspnea (NYHA)	3	3	3	0.1
CT-scan patterns (% patients, n=)				
NSIP	66	78(n=22)	56(n=19)	0.13
OP	5	4(n=1)	6(n=2)	0.88
UIP	8	0	14(n=5)	0.33
NSIP/OP	21	18(n=5)	24(n=8)	0.71

Q1-Q3: quartile1-quartile3; PFT: Pulmonary function test; FVC: forced vital capacity; FEV1: forced expiratory volume in 1seconde; TPC: Total pulmonary capacity; cDLCO: corrected diffusing capacity; NSIP: non specific interstitial pneumonia; OP: organized pneumonia; UIP: usual interstitial pneumonia.

Table 3: Patients features at follow up.

		Rituximab	Cyclophosphamide	*p*-value
PFTs				
Median FVC evolution at 6 months, (% of baseline)		+4.5 (-14;+34) n=22	+4 (-35;+65) n=33	0.46
Median cDLCO evolution at 6 months, (% of baseline)		+2 (-12 ;+19) n=21	+3 (-14 ;+28) n=32	0.86
Median FVC evolution at 1 year, (% of baseline)		+7 (-9;+30) n=24	+4 (-19 ;+37) n=19	0.18
Median cDLCO evolution at 1 year, (% of baseline)		+1 (-10 ;+21) n=24	+4 (-12 ;+34) n=17	0.61
Median FVC evolution at 2 years, (% of baseline)		+7 (-12;+34) n= 16	+5 (-13;+27) n=8	0.73
Median cDLCO evolution at 2 years, (% of baseline)		+5 (-10;+57) n=16	+3 (-4;+20) n=9	0.78
CT-scan				
Improvement of ILD extension		11/24	8/30	0.16
Worsening of ILD extension		1/24	8/30	**0.03**
Additional treatments (n=)	AZA	6	14	
	MTX	3	2	
	MMF	5	12	
	CalciNI	1	2	
	IGV	2	7	
	None	13	0	
Adverse events				
All infections		36%(n=10)	38%(n=13)	1.0
Severe infections		4%(n=1)	12%(n=4)	0.37
Death		4%(n=1)	3%(n=1)	1.0

PFTs: pulmonary function tests; FVC: forced vital capacity; cDLCO: corrected diffusing capacity. IVIG: intravenous immunoglobulin; AZA: Azathioprine; MTX: Methotrexate; MMF: mycophenolate mophetil; CalciNI : calcineurin-Inhibitor (cyclosporine or tacrolimus)

Table 4 : Bivariate and multivariate analyses at 2 years

	Bivariate HR [IC95]	p	Multivariate HR [IC95]	p
Rituximab	0.223 [0.084; 0.59]	0.003	0.263 [0.094; 0.732]	**0.011**
cDLCO baseline (%)	0.958 [0.93; 0.986]	0.004	0.964 [0.935; 0.994]	**0.019**
Previous number of IST at baseline ≥ 2	0.814 [0.381; 1.74]	0.60	1.226 [0.553; 2.718]	0.62

HR: Hazard ratio; cDLCO: corrected diffusing capacity

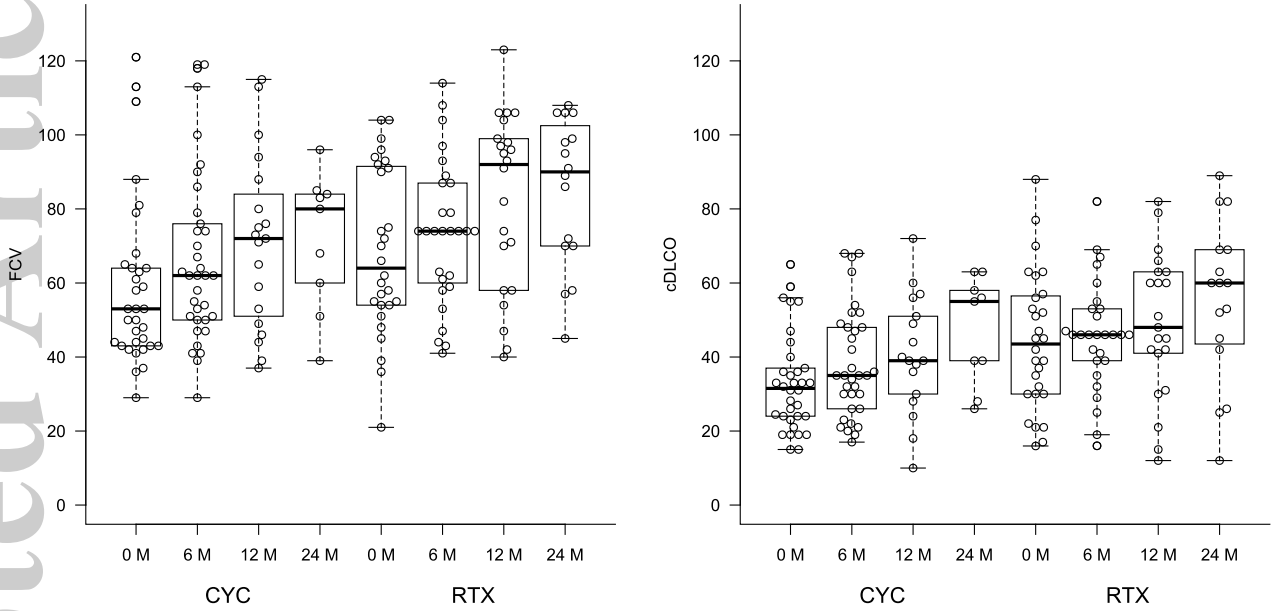

Figure 1 : Similar FVC and cDLCO evolution over time in CYC and RTX groups.
FVC: forced vital capacity; cDLCO: corrected diffusing capacity.

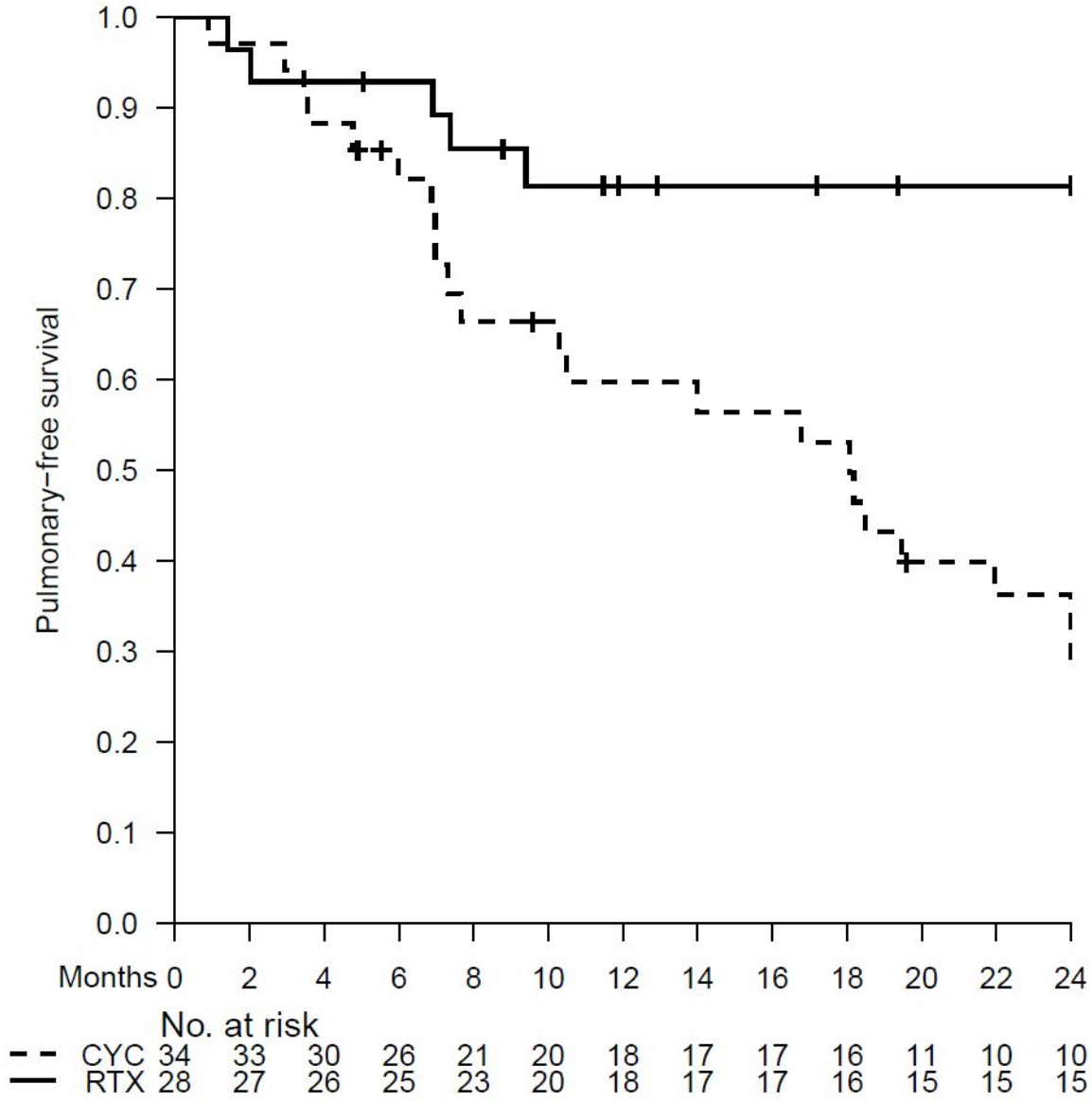

Figure 2 : Pulmonary free-survival curves after 2 years of follow-up.