Title

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Peripheral blood gene expression profiling shows predictive significance for response to mycophenolate in systemic sclerosis-related interstitial lung disease

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The study results have been presented at the American College of Rheumatology Convergence Meeting in 2021.[35]

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ABSTRACT

Objectives To characterise the peripheral blood cell (PBC) gene expression changes ensuing from mycophenolate mofetil (MMF) or cyclophosphamide (CYC) treatment and to determine the predictive significance of baseline PBC transcript scores for response to immunosuppression in systemic sclerosis (SSc)-related interstitial lung disease (ILD).

Methods PBC RNA samples from baseline and 12-month visits, corresponding to the active treatment period of both arms in Scleroderma Lung Study II, were investigated by global RNA sequencing. Joint models were created to examine the predictive significance of *baseline* composite modular scores for the course of forced vital capacity (FVC) per cent predicted measurements from 3 to 12 months.

Results 134 patients with SSc-ILD (CYC=69 and MMF=65) were investigated. CYC led to an upregulation of erythropoiesis, inflammation and myeloid lineage-related modules and a downregulation of lymphoid lineage-related modules. The modular changes resulting from MMF treatment were more modest and included a downregulation of plasmablast module. In the longitudinal analysis, none of the baseline transcript module scores showed predictive significance for FVC% course in the CYC arm. In contrast, in the MMF arm, higher baseline lymphoid lineage modules predicted better subsequent FVC% course, while higher baseline myeloid lineage and inflammation modules predicted worse subsequent FVC% course.

Conclusion Consistent with the primary mechanism of action of MMF on lymphocytes, patients with SSc-ILD with higher baseline lymphoid module scores had better FVC% course, while those with higher myeloid cell lineage activation score had poorer FVC% course on MMF.

Key messages

What is already known about this subject?

⇒ The immunosuppressive agent mycophenolate mofetil has become the most commonly used treatment for systemic sclerosis (SSc)-related interstitial lung disease (ILD).

⇒ However, response to immunosuppression (cyclophosphamide or mycophenolate mofetil) is highly variable in patients with this condition.

What does this study add?

⇒ Characterisation of peripheral blood cell gene expression changes resulting from immunosuppressive treatment indicated that oral cyclophosphamide has a profound impact on immune, coagulation and erythropoiesis-related modules, while mycophenolate leads to more modest gene expression changes, including a decline in the plasmablast module.

⇒ Consistent with the primary mechanism of action of mycophenolate on lymphocytes, patients with higher baseline lymphoid modules have better per cent predicted forced vital capacity (FVC%) course on mycophenolate, while those with higher myeloid cell lineage activation score have poorer FVC% course on mycophenolate.

How might this impact on clinical practice or future developments?

⇒ Peripheral blood cell gene expression profiling might identify patients with SSc-ILD who preferentially respond to mycophenolate mofetil.

⇒ With the emergence and development of novel therapeutics for SSc-ILD, peripheral blood cell gene expression profiling may improve our ability to personalise treatment for patients in the future.

INTRODUCTION

Interstitial lung disease (ILD) is the leading cause of disease-related death in systemic sclerosis (SSc).[1 2] Scleroderma Lung Studies (SLS) I[3] and II[4] showed that both cyclophosphamide (CYC) and mycophenolate mofetil (MMF) were effective in the treatment of SSc-ILD as measured by serially obtained per cent predicted forced vital capacity (FVC%). Moreover, the recently completed Safety and Efficacy of Nintedanib in Systemic Sclerosis (SENSCIS) trial provided supportive data on the efficacy of background therapy with MMF as monotherapy or in combination with nintedanib in SSc-ILD.[5] MMF is the most commonly used treatment for this disease manifestation in the clinical setting.[6] However, response to immunosuppression is highly variable in SSc-ILD, with approximately one-third of patients experiencing lung volume decline despite treatment in SLS I and SLS II studies.[3 4] Moreover, CYC and MMF can be associated with serious side effects, emphasising the need for identification of likely responders.[3 4 7] However, there

are presently no widely accepted clinical or laboratory markers that can reliably predict response to immunosuppression in SSc-ILD. The recent approvals of the antifibrotic agent nintedanib[8] and the anti-interleukin (IL)-6 agent tocilizumab[9] have expanded our treatment options for SSc-ILD, but have also further underscored the unmet clinical need for better predictive biomarkers that can inform the timely initiation of the most effective treatment and prevention of irreversible lung damage.

Contrary to lung tissue, peripheral blood cell (PBC) RNA can be obtained during routine clinical care. Moreover, the availability of approved storage systems such as PAXgene and Tempus tubes has the advantage of RNA being immediately stabilised after blood draw and not affected by well-documented gene expression changes due to transport and ex vivo handling,[10 11] enabling their use in clinical setting and multicentre clinical trials. Despite its potential for clinical use, there are no previous studies examining the predictive significance of PBC RNA for response to MMF or CYC in SSc-ILD. In regard to treatment-related molecular changes, we have previously reported on PBC gene expression changes resulting from intravenous monthly CYC in SSc in the Scleroderma: Cyclophosphamide or Transplantation (SCOT) trial,[12] but similar results have not been published for treatment with oral CYC. Moreover, there are no published reports on the impact of MMF treatment on the PBC gene expression profile of patients with SSc. Beyond its potential value as predictive biomarkers, characterisation of MMF-associated treatment effect at PBC gene expression level can provide useful molecular data for ongoing and future clinical trials in SSc, as the majority of them permit MMF background treatment.

Capitalising on the valuable PBC RNA samples collected in SLS II, we sought to characterise the PBC gene expression changes ensuing from MMF or CYC treatment and to determine the predictive significance of baseline PBC transcript scores for response to immunosuppressive treatment in SSc-ILD.

METHODS

Study participants

All SLS II patients with an available baseline PAXgene sample were included in the present study. The eligibility criteria for SLS II have been published previously[4] and key inclusion and exclusion criteria are listed in the online supplemental methods. Written informed consent was obtained from all study participants.

SLS II study design

Patients were randomised to receive either MMF for 2 years or oral CYC for 1 year followed by 1 year of placebo. Based on this design, both treatment arms were on active treatment during the first 12 months, while the participants in the MMF arm were continued on MMF therapy and those in the CYC arm were placed on placebo during the second year. Therefore, the present study focused on the analysis of gene expression changes during the first year of study during which both treatment arms were receiving active treatment. FVC% as continuous variable was the primary outcome and was measured every 3 months.

Gene expression profiling and analysis

Whole blood samples were collected in PAXgene tubes (BD Biosciences, Franklin Lakes, New Jersey) and stored at −80°C. PBC RNA was extracted according to the manufacturer's protocol. Global RNA sequencing was performed with Illumina NovaSeq 6000 (see online supplemental methods for further details). The gene expression data are deposited in the National Center for Biotechnology Information (NCBI)'s Gene Expression Omnibus.[13]

Modular analysis statistics

Modular analysis using 62 curated whole blood modules was conducted using the original repertoire analysis[14] (see online supplemental material). In addition to the traditional repertoire analysis based on the percentage of upregulated and downregulated transcripts within a module, a gene set analysis was conducted using the QuSAGE algorithm[15] for the modular analysis of differentially expressed genes. QuSAGE tests whether the average log2 fold change of a gene set is different from zero. The method correctly adjusts for gene-to-gene correlations within a gene set and provides an easily interpretable metric for the magnitude of differential regulation. A threshold value of false discovery rate (FDR) <0.05 *and* log2 fold change >0.2 was used to identify differentially expressed modules. The analysis of treatment-related changes compared each of the follow-up sample with its own baseline sample by employing a QuSAGE analysis based on linear mixed model, which took into account patient random effect. Moreover, a composite score was calculated for each module (see online supplemental methods for further details).

Determination of predictive significance of transcript modules

Joint models[16] combining a mixed effects model for the longitudinally obtained FVC% with a survival model to handle non-ignorable missing data due to study dropouts, treatment failure or death were used for each treatment arm. The joint models consisted of a linear mixed effects submodel examining FVC% from 3 to 12 months as continuous variable, with fixed effects for the baseline modular score, time (as a continuous variable in months) and baseline FVC%, with a random slope and intercept. The survival submodel was a Cox proportional hazards model predicting time to treatment discontinuation up until 12 months with terms for the modular score and baseline FVC%. Each baseline modular score (primary outcome variable of interest) was analysed in a separate model. P values for the baseline transcript score were adjusted for FDR to account for multiple comparisons, and modules with $p_{FDR<0.05}$ were defined as having predictive significance.

In an exploratory analysis, responder analyses were also performed. FVC% cut-off values previously developed based on the pooled SLS I and II data were used to define response,[17] in which the minimal clinically important difference (MCID) for improvement was an increase in FVC% >3% and the MCID for worsening was defined as FVC% decline <−3%. The FVC% measurement at 12 months compared with baseline visit was used in this analysis. In six patients with an available 9-month but missing 12-month visit measurement, the 9-month FVC% was carried forward. Logistical regression was used to determine the predictive significance of baseline modular scores for response status. Considering the loss of power with dichotomising a continuous outcome variable and the exploratory nature of this analysis, this analysis was not corrected for multiple comparison.

Patient and public involvement

This research was funded in part by the Department of Defense (DoD) Congressionally Directed Medical Research Programs, which included patient representatives in their review panels.

RESULTS

Among 142 enrolled patients, PBC RNA samples of sufficient quantity and quality for global gene expression profiling were

Table 1 Baseline patient characteristics

Characteristic	CYC, n=69	MMF, n=65	Overall, n=134
Age in years*	52.0±9.5	52.8±9.9	52.4±9.7
Female, n (%)	53 (76.8)	45 (69.2)	98 (73.1)
Race, n (%)			
White	45 (65.2)	48 (73.8)	93 (69.4)
African American	18 (26.1)	11 (16.9)	29 (21.6)
Asian	3 (4.3)	6 (9.2)	9 (6.7)
Native American	3 (4.3)	0 (0.0)	3 (2.2)
Hispanic ethnicity, n (%)	9 (13.0)	8 (12.3)	17 (12.7)
Diffuse disease type, n (%)	39 (56.5)	40 (61.5)	79 (59.0)
Disease duration in years*	2.5±1.8	2.8±1.8	2.6±1.8
FVC%*	66.0±9.9	66.5±8.2	66.3±9.1
DLCO %*	54.3±14.1	54.3±11.3	54.3±12.8
mRSS*	14.3±10.8	15.1±10.2	14.7±10.5
Antitopoisomerase I, n (%)†	30 (44.1)	28 (45.2)	58 (44.6)
Anti-RNA polymerase III, n (%)†	8 (11.8)	9 (14.5)	17 (13.1)

*Mean±SD.
†Antibody data are missing in four participants.
CYC, cyclophosphamide; DLCO%, per cent predicted diffusing capacity for carbon monoxide; FVC%, per cent predicted forced vital capacity; MMF, mycophenolate mofetil; mRSS, modified Rodnan Skin Score.

available in 134 patients at baseline (CYC=69 and MMF=65) and in 98 patients (CYC=47 and MMF=51) at the 12-month visit. As shown in table 1, baseline patient characteristics were balanced between patients assigned to CYC and MMF arms. The mean disease duration was 2.6 years and 59% of patients had diffuse cutaneous involvement in the overall patient population.

Transcript-level gene expression changes after treatment

Treatment with oral CYC led to substantial changes in PBC gene expression profile. Specifically, 6873 transcripts were differentially expressed after treatment with CYC in comparison with baseline samples. The effect of MMF on PBC gene expression was more modest, as reflected by the fact that only 113 transcripts were differentially expressed after treatment with MMF as determined by the pairwise comparison of 12-month visit samples with the baseline samples. An Ingenuity Pathway Analysis indicated that the top over-represented canonical pathways in the CYC arm were phagosome formation, ferroptosis signalling and hepatic fibrosis signalling, while the top canonical pathways in the MMF arm were primary immunodeficiency signalling, kinetochore metaphase signalling and B cell receptor signalling.

Modular gene expression changes after treatment

A previously described modular analysis method was completed.[12 14 18 19] In this analysis, 62 gene expression modules (sets of coexpressed genes) that are observed in whole blood across a variety of inflammatory and infectious diseases were investigated. Where possible, a biological function was assigned to a module based on the function of genes present in this module (eg, myeloid lineage, T cell, etc), and these modules are called annotated modules. Other modules remained uncategorised (not annotated).

The comparison of baseline samples in the CYC arm with baseline samples in the MMF arm did not yield any significant differentially expressed modules, indicating that randomisation was successful in avoiding molecular differences between the two treatment arms at enrolment (online supplemental table 1).

Table 2 Results of QuSAGE analysis for differentially expressed annotated modules in pairwise comparison of 12-month with baseline samples in the CYC arm

Module	Annotation	Log2 fold change	P_{FDR} value
M2.3	Erythropoiesis	1.21	<0.0001
M6.18	Erythropoiesis	0.93	<0.0001
M3.1	Erythropoiesis	0.91	<0.0001
M4.4	Erythropoiesis	0.57	<0.0001
M5.15	Neutrophils/granulocytes	0.53	<0.0001
M4.2	Inflammation	0.47	<0.0001
M5.3	Erythropoiesis	0.39	<0.0001
M1.1	Coagulation/platelets	0.35	0.002
M3.3	Cell cycle/proliferation	0.34	<0.0001
M3.2	Myeloid lineage	0.31	<0.0001
M6.11	Cell cycle/proliferation	0.28	0.0042
M4.14	Monocytes	0.27	<0.0001
M6.14	Coagulation/platelets	0.26	0.0001
M6.6	Myeloid lineage	0.26	<0.0001
M3.4	IFN response	0.24	0.0103
M4.6	Myeloid lineage	0.23	0.0001
M4.13	Inflammation	0.21	0.0055
M6.13	Inflammation	0.2	<0.0001
M3.6	Cytotoxic/NK cell	−0.23	0.0095
M4.3	Protein synthesis	−0.25	0.0021
M6.12	Lymphoid lineage	−0.26	<0.0001
M4.7	Lymphoid lineage	−0.3	<0.0001
M6.9	Lymphoid lineage	−0.36	<0.0001
M4.15	Cytotoxic/NK cell	−0.46	<0.0001
M6.15	T cells	−0.51	<0.0001
M6.19	T cells	−0.62	<0.0001
M4.1	T cells	−0.82	<0.0001
M4.11	Plasmablasts	−0.98	<0.0001
M4.10	B cells	−1.29	<0.0001

CYC, cyclophosphamide; FDR, false discovery rate; IFN, Interferon; NK, Natural Killer.

As listed in table 2 and shown in figure 1, the pairwise comparison of 12 months with baseline samples showed an upregulation of erythropoiesis, inflammation and myeloid lineage-related modules and a downregulation of lymphoid lineage-related modules in the CYC arm.

Consistent with the transcript-level analysis, the modular changes ensuing from MMF treatment were more modest. As shown in table 3 and figure 1, plasmablast and cell cycle modules were downregulated after MMF treatment.

Predictive significance of modular gene expression for the course of FVC

Next, composite scores were calculated for the gene expression modules shown in figure 1. Online supplemental tables 2 and 3 show the correlation/association of baseline modular scores with baseline disease duration, FVC% and modified Rodnan Skin Score (mRSS), as well as disease type and antitopoisomerase I/RNApolymerase III positivity. In this cross-sectional analysis, none of the baseline gene expression module scores was associated with baseline disease characteristics after correction for multiple comparison. Moreover, the baseline gene expression modules scores did not predict the course of mRSS during the 3-month to 12-month follow-up visits in the MMF or CYC arm (this analysis was confined to patients with diffuse cutaneous involvement) (online supplemental table 4).

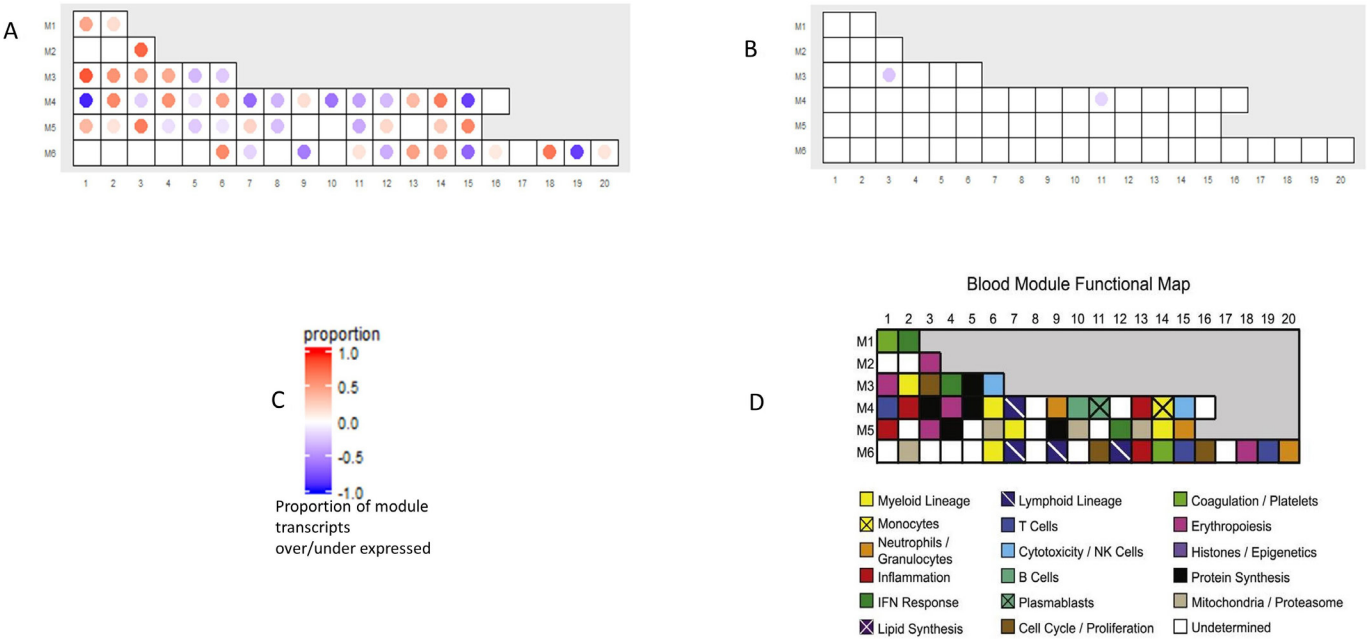

Figure 1 Differentially expressed modules in pairwise comparisons of 12-month visit with baseline SSc samples in the CYC (A) and MMF (B) arms based on traditional repertoire analysis (the percentage of upregulated and downregulated transcripts within a module). (C) Legend for the colour coding in A and B. (D) Annotation of modules based on known biological function of genes included in a given module. The numbers on y and x axes indicate the main module and submodule designation, respectively. Of note, the module map in this figure and the results in tables 2 and 3 are based on two different analytic algorithms (repertoire analysis vs QuSAGE). CYC, cyclophosphamide; MMF, mycophenolate mofetil; NK, natural killer; SSc, systemic sclerosis.

Next, the predictive significance of gene expression module scores for the course of FVC% during the 3-month to 12-month follow-up period was investigated. None of the baseline module scores significantly predicted the course of FVC% during this period in the CYC arm (online supplemental table 5). In contrast, as shown in figure 2 and listed in table 4, in the MMF arm, higher baseline lymphoid lineage (including T cells and cytotoxic/natural killer (NK) cells), as well as mitochondrial and protein synthesis modules, showed predictive significance for a better subsequent FVC% course, while higher baseline myeloid lineage (including neutrophils/granulocytes) and inflammation modules showed predictive significance for a worse subsequent FVC% course. For example, a one-unit higher baseline lymphoid lineage modular score (corresponding to an increase of one unit in the averaged Z-scores of transcript contained in the module) was associated with 2.85% higher FVC% during the 3-month to 12-month visits. A complete list of transcript modules and their predictive significance is provided in online supplemental table 5.

In an exploratory responder analysis based on previously defined MCID values,[17] 52 participants were defined as improvers (FVC% increase >3%), while 64 participants were categorised as non-improvers. Consistent with the primary analysis, patients with higher lymphoid lineage and mitochondrial module scores were more likely to have FVC% improvement, while those with higher myeloid lineage, neutrophil/granulocyte and inflammation modules were less likely to have an improvement in the MMF arm (table 5). For example, a one-unit increase in the lymphoid module score predicted 3.6 times higher likelihood of having an improvement in FVC% in the MMF arm.

Table 3 Results of QuSAGE analysis for differentially expressed annotated modules in pairwise comparison of 12-month with baseline samples in the MMF arm

Module	Annotation	Log2 fold change	P_{FDR} value
M3.3	Cell cycle	−0.43	<0.0001
M6.11	Cell cycle/DNA repair	−0.39	0.0003
M4.11	Plasmablast	−0.77	<0.0001

FDR, false discovery rate; MMF, mycophenolate mofetil.

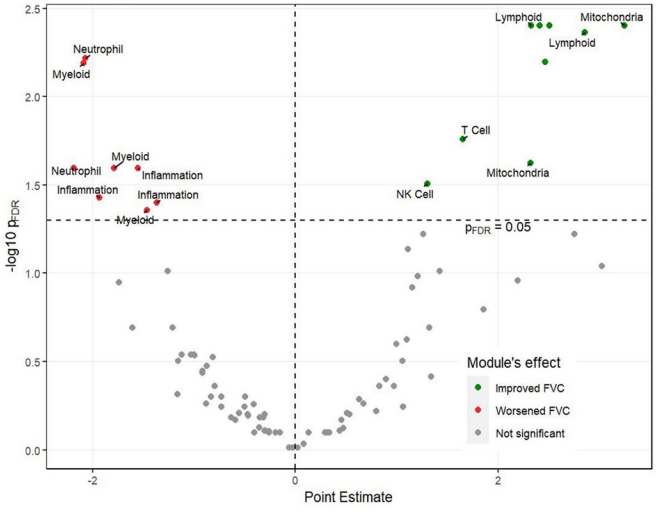

Figure 2 Predictive significance of baseline modular scores for FVC% during visits at 3–12 months in the MMF arm. Higher lymphoid module scores showed predictive significance for better ILD course, while higher neutrophil/myeloid lineage module scores showed predictive significance for worse ILD course. Of note, the modular analysis method can assign the same biological function to multiple modules. All annotated modules in figure 1 are included in this figure. FDR, false discovery rate; FVC%, per cent predicted forced vital capacity; ILD, interstitial lung disease; MMF, mycophenolate mofetil.

Table 4 Baseline annotated modular scores that showed predictive significance for the course of FVC% (as a continuous variable) during the 3-month to 12-month visits in the MMF arm*†

Module	Annotation	Point estimate	95% CI	P_{FDR} value
M5.10	Mitochondria/ proteasome	3.24	1.55 to 4.94	0.00396
M6.12	Lymphoid lineage	2.85	1.33 to 4.38	0.00434
M3.5	Protein synthesis	2.51	1.26 to 3.75	0.00396
M5.9	Protein synthesis	2.46	1.08 to 3.84	0.00639
M4.3	Protein synthesis	2.41	1.2 to 3.62	0.00396
M6.9	Lymphoid lineage	2.33	1.12 to 3.53	0.00396
M5.6	Mitochondria/ proteasome	2.32	0.8 to 3.83	0.02371
M6.19	T cells	1.65	0.61 to 2.69	0.01746
M4.15	Cytotoxic/NK cell	1.3	0.4 to 2.2	0.03106
M4.2	Inflammation	−1.37	−2.35 to −0.38	0.04002
M3.2	Myeloid lineage	−1.46	−2.53 to −0.39	0.04383
M4.13	Inflammation	−1.56	−2.6 to −0.51	0.02545
M5.14	Myeloid lineage	−1.79	−2.98 to −0.59	0.02545
M5.1	Inflammation	−1.93	−3.3 to −0.56	0.03735
M6.20	Neutrophils/ granulocytes	−2.07	−3.21 to −0.92	0.00604
M5.7	Myeloid lineage	−2.09	−3.26 to −0.91	0.00649
M4.9	Neutrophils/ granulocytes	−2.18	−3.65 to −0.72	0.02545

*Each included the listed module score (each module score separately), baseline FVC% and time as independent variables.
†Four additional not annotated modulates (M2.1, M4.12, M5.5, M6.3) showed predictive significance for the course of FVC in the MMF arm (see online supplemental table 5 for additional details).
FDR, false discovery rate; FVC%, per cent predicted forced vital capacity; MMF, mycophenolate mofetil.

In the FVC% worsening analysis (FVC% decline <−3%), 26 participants were defined as having worsening, while 90 were categorised as non-decliners. Consistent with the primary analysis, higher myeloid lineage and inflammation module scores predicted an FVC% worsening, while higher lymphoid lineage, T cell and mitochondrial modules had lower likelihood of an FVC% worsening in the MMF arm (table 6). Of note, consistent with primary analysis, none of the baseline modular scores predicted FVC% improvement or worsening in the responder analysis in the CYC arm (data not shown).

Table 5 Predictive significance of baseline annotated modular scores for improvement in FVC% (as a dichotomised variable) at 12 months in the MMF arm

Module	Annotation	OR	95% CI	P value
M5.10	Mitochondria/proteasome	3.68	1.09 to 12.44	0.0358
M6.12	Lymphoid lineage	3.63	1.21 to 10.89	0.0215
M6.9	Lymphoid lineage	2.9	1.16 to 7.26	0.0233
M4.3	Protein synthesis	2.23	1.05 to 4.71	0.0359
M3.2	Myeloid lineage	0.48	0.24 to 0.98	0.0444
M4.13	Inflammation	0.46	0.23 to 0.92	0.0277
M5.7	Myeloid lineage	0.4	0.17 to 0.92	0.0313
M6.20	Neutrophils/granulocytes	0.36	0.16 to 0.8	0.0124
M5.14	Myeloid lineage	0.35	0.16 to 0.78	0.0105

FVC%, per cent predicted forced vital capacity; MMF, mycophenolate mofetil.

Table 6 Predictive significance of baseline annotated modular scores for worsening in FVC% (as a dichotomised variable) at 12 months in the MMF arm

Module	Annotation	OR	95% CI	P value
M5.7	Myeloid lineage	5.18	1.6 to 16.78	0.006
M5.14	Myeloid lineage	4.86	1.54 to 15.29	0.0069
M5.1	Inflammation	4.65	1.43 to 15.13	0.0107
M6.20	Neutrophils/granulocytes	4.62	1.63 to 13.05	0.0039
M4.9	Neutrophils/granulocytes	3.95	1.2 to 12.99	0.0239
M4.13	Inflammation	3.36	1.36 to 8.27	0.0084
M6.13	Inflammation	3.22	1.07 to 9.74	0.0381
M3.2	Myeloid lineage	2.76	1.19 to 6.37	0.0177
M4.2	Inflammation	2.26	1.09 to 4.66	0.0275
M4.6	Myeloid lineage	2.25	1.05 to 4.79	0.0363
M4.11	Plasmablasts	0.43	0.2 to 0.97	0.0414
M6.19	T cells	0.43	0.19 to 0.96	0.04
M4.10	B cells	0.42	0.2 to 0.91	0.0267
M4.15	Cytotoxic/NK cell	0.42	0.2 to 0.84	0.0153
M6.15	T cells	0.40	0.17 to 0.96	0.041
M4.3	Protein synthesis	0.38	0.16 to 0.9	0.0281
M3.6	Cytotoxic/NK cell	0.36	0.13 to 0.98	0.0457
M5.6	Mitochondria/proteasome	0.31	0.11 to 0.91	0.0325
M3.5	Protein synthesis	0.26	0.09 to 0.69	0.0074
M6.9	Lymphoid lineage	0.25	0.09 to 0.7	0.0081
M5.10	Mitochondria/proteasome	0.20	0.05 to 0.78	0.0205
M6.12	Lymphoid lineage	0.19	0.05 to 0.64	0.0073
M6.16	Cell cycle/DNA repair	0.11	0.02 to 0.64	0.0132

FVC%, per cent predicted forced vital capacity; MMF, mycophenolate mofetil; NK, Natural Killer.

DISCUSSION

In the present study, PBC gene expression changes ensuing from CYC or MMF in patients enrolled in the SLS II were examined, showing that oral CYC had a profound impact on immune, coagulation and erythropoiesis-related modules, while MMF led to more modest gene expression changes, including a decline in the plasmablast module. We also studied the predictive significance of PBC transcript profile for response to immunosuppression in SSc-ILD, showing that patients with higher baseline lymphoid modules had better FVC% course, while those with higher myeloid cell lineage activation score had poorer FVC% course on MMF.

CYC alkylates DNA and thereby inhibits cell division. In our previous study in the SCOT trial, intravenous monthly CYC treatment led to a decline in the B cell module (4.10) and an increase in the neutrophil (5.15) module. These changes were also observed in the present study among patients treated with oral CYC. However, oral CYC also led to significant increases in the erythropoiesis, coagulation and myeloid lineage immune modules, as well as decreases in the lymphoid lineage modules. The more profound impact of CYC on the PBC gene expression profile in SLS II than in the SCOT trial may be due to higher sample size in the present study or differences in dosage and mode of administration (oral daily vs intravenous monthly). A differential impact of CYC on PBCs based on the mode of administration is supported by a recent study showing a four times higher cumulative dose of CYC and higher frequency of leucopenia with the daily oral than with the intravenous CYC administration in patients with SSc.[20] Moreover, oral CYC had a more profound impact on PBC gene expression profile than MMF in the present study. This is consistent with the clinical

observation that CYC had a worse tolerability and toxicity profile than MMF in SLS II.[4]

MMF is a prodrug of mycophenolic acid. Mycophenolic acid preferentially impairs guanosine nucleotide synthesis in T and B lymphocytes by blocking the enzyme IMPDH (inosine-5-monophosphate dehydrogenase) because it is five times more potent in inhibiting the type II isoform of this enzyme, which is expressed in activated T and B lymphocytes than its housekeeping isoform (type I), which is expressed in most cell types.[21 22] Consistent with the primary mechanism of action of MMF on lymphocytes, we observed that MMF treatment led to a decline in the plasmablast transcript module and that patients with a high lymphoid lineage gene expression profile had a better response to MMF. While there are no other published data on PBC gene expression or flow cytometry-based immune cell count changes ensuing from MMF treatment in SSc, a decline in the number of peripheral blood plasmablasts, B cells and T cells has been reported in cytometry-based studies in systemic lupus erythematosus.[23 24] The MMF-mediated downregulation of plasmablast function[23] might also explain the results of a recent study of 686 patients with autoimmune rheumatic diseases indicating MMF (along with rituximab and abatacept) treatment was associated with a significantly reduced response to COVID-19 vaccine.[25] Specifically, the seropositivity rate of patients treated with MMF was 64%, while patients treated with methotrexate, leflunomide, anti-tumour necrosis factor (TNF) and anti-IL-6 monotherapies had immune responses above 90%. Similarly, patients with solid organ transplant recipients who are treated with MMF were at higher risk of mounting an insufficient response to COVID-19 vaccination.[26–28]

A global gene expression study examining the transcript changes ensuing from MMF treatment in SSc skin reported a decline in T cell, activated dendritic cell and macrophage transcript modules based on the longitudinal assessment of six patients but not in B cell modules following treatment.[29] The discrepancy between the skin findings in the previous study and our PBC transcript results might stem from differences in SSc immune signatures at the PBC and end-organ level. Specifically, the immune signature in SSc skin is influenced by abundance of specific cell types, homing of immune cells from blood into the affected tissue and the local inflammatory cytokine milieu. For example, low abundance of B cells in the skin tissue might have contributed to the lack of B cell signature changes in the aforementioned study. Moreover, MMF can inhibit the glycosylation and function of adhesion molecules, resulting in decreased extravasation of T cells and monocytes into the affected tissue, which might in part explain the observed decline of T cell, activated dendritic cell and macrophage transcript modules in SSc skin following MMF treatment.[21 30 31]

In the present study, neither CYC nor MMF led to a decrease in the main interferon module (M1.2), which was previously reported to be the most upregulated transcript module in SSc PBCs.[12] This is consistent with our findings in the SCOT trial, in which intravenous monthly CYC, contrary to haematopoietic stem cell transplantation, did not lead to a decline in this module.[12] However, we have recently reported a composite score of 6 serum interferon inducible proteins decreased (but not normalised) with CYC and MMF treatment in SLS II.[32] This discrepancy between PBC RNA and serum protein results might stem from the fact that serum proteins are also influenced by a spillover effect from affected end organs. In fact, two recent studies indicated that the differential expression for most serum proteins in SSc was likely to originate outside PBCs.[33 34]

Our study has several strengths. Capitalising on the standardised, uniform treatment protocols in SLS II, we characterised for the first time the PBC gene expression changes ensuing from MMF treatment in SSc. Moreover, we examined for the first time the predictive significance of PBC transcripts for response to treatment in SSc-ILD, showing patients with high lymphoid lineage module scores had better FVC% course on MMF, raising the possibility that PBC gene expression profiling can potentially identify patients who would preferentially benefit from MMF. It would be informative to extend these PBC gene expression studies to valuable samples collected in the recently completed SSc-ILD trials of antifibrotic (nintedanib)[8] and anti-IL-6 (tocilizumab) agents,[9] with the ultimate goal of developing prediction models that inform the timely initiation of the most effective treatment modality.

The present study also has limitations. SLS II did not include a placebo arm during the first year of the study period. Therefore, we cannot investigate the predictive significance of aforementioned transcript modules in untreated patients with SSc-ILD. However, it is likely the observed predictive significance of baseline immune modules in the MMF arm is related to treatment effect (vs the natural history of SSc-ILD) as the same modules did not predict ILD course in the CYC arm. Moreover, the baseline PBC gene expression modules did not show predictive significance for the course of mRSS, which might be due to the fact that SLS II did not include sufficient number of patients with progressive skin involvement. It would be informative to investigate the predictive significance of the PBC modular scores for the mRSS course in future trials that are enriched for skin fibrosis progressors.

In conclusion, oral CYC has a profound impact on the PBC gene expression profile in patients with SSc-ILD, potentially accounting for the higher known toxicity.[4 20] MMF treatment leads to more modest gene expression changes, including a decline in the plasmablast module. The baseline PBC immune modules showed predictive significance for the course of SSc-ILD in the MMF arm. Consistent with the primary mechanism of action of MMF on lymphocytes,[21] patients with higher baseline lymphoid module scores had better FVC% course, while those with higher myeloid cell lineage activation score had poorer FVC% course on MMF. With the emergence and development of novel therapeutics for SSc-ILD, gene expression profiling may improve our ability to personalise treatment for patients in the future.

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REFERENCES

1 Steen VD, Medsger TA. Changes in causes of death in systemic sclerosis, 1972-2002. *Ann Rheum Dis* 2007;66:940–4.
2 Tyndall AJ, Bannert B, Vonk M, *et al*. Causes and risk factors for death in systemic sclerosis: a study from the EULAR scleroderma trials and research (EUSTAR) database. *Ann Rheum Dis* 2010;69:1809–15.
3 Tashkin DP, Elashoff R, Clements PJ, *et al*. Cyclophosphamide versus placebo in scleroderma lung disease. *N Engl J Med* 2006;354:2655–66.
4 Tashkin DP, Roth MD, Clements PJ, *et al*. Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): a randomised controlled, double-blind, parallel group trial. *Lancet Respir Med* 2016;4:708–19.
5 Highland KB, Distler O, Kuwana M, *et al*. Efficacy and safety of nintedanib in patients with systemic sclerosis-associated interstitial lung disease treated with mycophenolate: a subgroup analysis of the SENSCIS trial. *Lancet Respir Med* 2021;9:96–106.
6 Jaafar S, Lescoat A, Huang S, *et al*. Clinical characteristics, visceral involvement, and mortality in at-risk or early diffuse systemic sclerosis: a longitudinal analysis of an observational prospective multicenter US cohort. *Arthritis Res Ther* 2021;23:170.
7 Radis CD, Kahl LE, Baker GL, *et al*. Effects of cyclophosphamide on the development of malignancy and on long-term survival of patients with rheumatoid arthritis. A 20-year followup study. *Arthritis Rheum* 1995;38:1120–7.
8 Distler O, Highland KB, Gahlemann M, *et al*. Nintedanib for systemic sclerosis-associated interstitial lung disease. *N Engl J Med* 2019;380:2518–28.
9 Khanna D, Lin CJF, Furst DE, *et al*. Tocilizumab in systemic sclerosis: a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Respir Med* 2020;8:963–74.
10 Rainen L, Oelmueller U, Jurgensen S, *et al*. Stabilization of mRNA expression in whole blood samples. *Clin Chem* 2002;48:1883–90.
11 Baechler EC, Batliwalla FM, Karypis G, *et al*. Expression levels for many genes in human peripheral blood cells are highly sensitive to ex vivo incubation. *Genes Immun* 2004;5:347–53.
12 Assassi S, Wang X, Chen G, *et al*. Myeloablation followed by autologous stem cell transplantation normalises systemic sclerosis molecular signatures. *Ann Rheum Dis* 2019;78:1371–8.
13 Assassi S, Volkmann ER, Zheng WJ. Peripheral blood gene expression profiling shows predictive significance for response to mycophenolate in systemic sclerosis related interstitial lung disease., 2021. Gene expression Omnibus (GEO). Available: https://urldefense.proofpoint.com/v2/url?u=https-3A__www.ncbi.nlm.nih.gov_geo_query_acc.cgi-3Facc-3DGSE181228&d=DwIBAg&c=bKRySV-ouEg_AT-w2QWsTdd9X__KYh9Eq2fdmQDVZgw&r=qvy3-zelO2xNSq2NIjNTOvegGZReJKZvukeNZcmKRkE&m=oFa6Zkj5NciSw4u4YGwWmyes5ASAXQGqLtzq8ls-rTc&s=QSEyHIqPy79zvJ1D20BhMFvG24x9MsJzC4aUlaqpZuE&e=
14 Chaussabel D, Baldwin N. Democratizing systems immunology with modular transcriptional repertoire analyses. *Nat Rev Immunol* 2014;14:271–80.
15 Yaari G, Bolen CR, Thakar J, *et al*. Quantitative set analysis for gene expression: a method to quantify gene set differential expression including gene-gene correlations. *Nucleic Acids Res* 2013;41:e170.
16 Li N, Elashoff RM, Li G. Robust joint modeling of longitudinal measurements and competing risks failure time data. *Biom J* 2009;51:19–30.
17 Kafaja S, Clements PJ, Wilhalme H, *et al*. Reliability and minimal clinically important differences of forced vital capacity: results from the scleroderma lung studies (SLS-I and SLS-II). *Am J Respir Crit Care Med* 2018;197:644–52.
18 Assassi S, Mayes MD, Arnett FC, *et al*. Systemic sclerosis and lupus: points in an interferon-mediated continuum. *Arthritis Rheum* 2010;62:589–98.
19 Banchereau R, Hong S, Cantarel B, *et al*. Personalized Immunomonitoring uncovers molecular networks that stratify lupus patients. *Cell* 2016;165:551–65.
20 Bruni C, Tashkin DP, Steen V, *et al*. Intravenous versus oral cyclophosphamide for lung and/or skin fibrosis in systemic sclerosis: an indirect comparison from EUSTAR and randomised controlled trials. *Clin Exp Rheumatol* 2020;38 Suppl 125:161–8.
21 Allison AC, Eugui EM. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology* 2000;47:85–118.
22 Carr SF, Papp E, Wu JC, *et al*. Characterization of human type I and type II IMP dehydrogenases. *J Biol Chem* 1993;268:27286–90.
23 Fassbinder T, Saunders U, Mickholz E, *et al*. Differential effects of cyclophosphamide and mycophenolate mofetil on cellular and serological parameters in patients with systemic lupus erythematosus. *Arthritis Res Ther* 2015;17:92.
24 Slight-Webb S, Guthridge JM, Chakravarty EF, *et al*. Mycophenolate mofetil reduces STAT3 phosphorylation in systemic lupus erythematosus patients. *JCI Insight* 2019;4. doi:10.1172/jci.insight.124575. [Epub ahead of print: 24 Jan 2019].
25 Furer V, Eviatar T, Zisman D, *et al*. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in adult patients with autoimmune inflammatory rheumatic diseases and in the general population: a multicentre study. *Ann Rheum Dis* 2021;80:1330–8.
26 Grupper A, Rabinowich L, Schwartz D, *et al*. Reduced humoral response to mRNA SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients without prior exposure to the virus. *Am J Transplant* 2021;21:2719–26.
27 Peled Y, Ram E, Lavee J, *et al*. BNT162b2 vaccination in heart transplant recipients: clinical experience and antibody response. *J Heart Lung Transplant* 2021;40:759–62.
28 Rabinowich L, Grupper A, Baruch R, *et al*. Low immunogenicity to SARS-CoV-2 vaccination among liver transplant recipients. *J Hepatol* 2021;75:435–8.
29 Hinchcliff M, Toledo DM, Taroni JN, *et al*. Mycophenolate mofetil treatment of systemic sclerosis reduces myeloid cell numbers and attenuates the inflammatory gene signature in skin. *J Invest Dermatol* 2018;138:1301–10.
30 Allison AC, Kowalski WJ, Muller CJ, *et al*. Mycophenolic acid and brequinar, inhibitors of purine and pyrimidine synthesis, block the glycosylation of adhesion molecules. *Transplant Proc* 1993;25:67–70.
31 Heemann U, Azuma H, Hamar P, *et al*. Mycophenolate mofetil inhibits lymphocyte binding and the upregulation of adhesion molecules in acute rejection of rat kidney allografts. *Transpl Immunol* 1996;4:64–7.
32 Assassi S, Li N, Volkmann ER, *et al*. Predictive significance of serum interferon-inducible protein score for response to treatment in systemic sclerosis-related interstitial lung disease. *Arthritis Rheumatol* 2021;73:1005–13.
33 Bellocchi C, Ying J, Goldmuntz EA, *et al*. Large-Scale characterization of systemic sclerosis serum protein profile: comparison to peripheral blood cell transcriptome and correlations with Skin/Lung fibrosis. *Arthritis Rheumatol* 2021;73:660–70.
34 Farutin V, Kurtagic E, Pradines JR, *et al*. Multiomic study of skin, peripheral blood, and serum: is serum proteome a reflection of disease process at the end-organ level in systemic sclerosis? *Arthritis Res Ther* 2021;23:259.
35 Assassi S, Zheng W, Volkmann E. Peripheral Blood Cell Gene Expression Profiling Predicts Response to Mycophenolate in Systemic Sclerosis Related Interstitial Lung Disease [abstract]. *Arthritis Rheumatol* 2021;73.